ELUCIDATING FECAL CONTAMINATION EXPOSURE IN LOW-INCOME COUNTRIES, THE CONTRIBUTION FROM CHILD FECES DISPOSAL PRACTICES AND SOIL INGESTION, AND LINKS TO CHILD HEALTH

BY

VALERIE BAUZA

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Environmental Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2017

Urbana, Illinois

Doctoral Committee:

Assistant Professor Jeremy S. Guest, Chair
Associate Professor Thanh H. Nguyen
Professor Joanna L. Shisler
Professor Assata Zerai

ABSTRACT

Enteric pathogens transmitted via fecal-oral pathways cause enteric infections that have substantial health and human capital consequences, making it critical to reduce child exposure to fecal contamination. Current water, sanitation, and hygiene programs in low-income countries often focus on improving water delivery and toilet/latrine infrastructure to reduce pathogen exposure, but child exposure to fecal contamination can remain common after these types of improvements. The overarching goal of this research was to investigate fecal contamination and enteric pathogen transmission in low-income countries, with a focus on young children's feces as a source of contamination, soil ingestion as an exposure point, and their effects on child health.

First, the occurrence, magnitude, and distribution of fecal contamination and enteric pathogens were assessed along multiple transmission pathways for children in a densely-populated urban slum neighborhood of Nairobi, Kenya. There was a high frequency of pathogen detection at several exposure points (including stored drinking water, hands, tables, plates, floors, soil, standing water, open drainage ditches, and streams) despite all households having access to a toilet or latrine. The results also provided evidence that children were exposed to enteric pathogens from several exposure points simultaneously, that there were interactions between different transmission pathways, and that soil could be an important exposure point because of its high levels of enteric pathogens.

Next, the role of poor child feces management practices for young children (who are not old enough to use a toilet facility themselves) was evaluated in the context of domestic fecal contamination and child health. A method to track fecal contamination from the feces of young children separately from older children/adults was developed, validated, and then used to analyze

environmental samples collected from multiple exposure points inside and outside households. Young children's feces dominated the human fecal contamination found in the majority of samples taken from the indoor environment (caregiver and child hands, tables, plates), older child/adult feces dominated the human fecal contamination found in the majority of samples taken from standing water and streams in the outdoor environment, and each source dominated the human fecal contamination found in an equal number of samples taken from open drainage ditches. These results provided evidence that young children's feces substantially contribute to household fecal contamination. Next, nationally representative data from 34 low- and middle-income countries was used to evaluate associations between child feces disposal practices and child health. Disposal of child feces into an improved toilet was found to be strongly associated with improvements in child growth, suggesting that better child feces disposal practices could achieve greater child health benefits than only improving toilet access.

Finally, this research investigated soil ingestion as a potential exposure pathway for fecal contamination. There were strong associations between soil ingestion and child diarrhea in an urban slum setting in Kenya and a rural setting in northern Ghana, despite high levels of finished floor in households in both settings. There was also a high prevalence of soil ingestion among children in both settings, indicating this is likely a common exposure pathway for children in low-income countries. Taken together, this work identified high levels of enteric pathogen contamination at numerous indoor and outdoor exposure points in an urban slum environment, performed detailed investigations of poor management of young children's feces as a contamination source and soil ingestion as an exposure point, and linked both of these practices to negative health consequences in children.

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Jeremy Guest, for being supportive of me throughout my PhD, helping me develop into a better researcher, and encouraging me pursue research related to my passions. I would also like to thank Jeremy for trusting my research ideas and using his start-up funds to try them out, even when we didn't know if they would work. I would like to thank Helen Nguyen, my co-advisor, for her feedback on my research as well as helping me improve my lab skills and become a more confident researcher. I am also sincerely grateful to Joanna Shisler and Assata Zerai for serving on my doctoral committee and providing helpful feedback on my dissertation. I would also like to thank Juliet Iwelunmor, Benito Mariñas, and Julie Zilles who served on my committee during the qualifying and/or preliminary exam phases, and provided helpful feedback related to my research direction.

I would also like to thank everyone who contributed to the fieldwork in Kenya included in this dissertation. First, I am grateful for Robinson Ocharo and Rodah Oboni, who made this work possible by selecting an appropriate field site and coordinating the fieldwork with local community members. I am also grateful for their assistance with household surveys. I would also like to give a huge thanks to Vincent Madadi, who ensured access to the lab space and equipment necessary to perform this work. Additionally, I would like to thank Phantus Wambiya and Philip Omandid for their help with household surveys and sample collection. Finally, I would like to thank Gabi Levato for her exceptional help with sample collection and daily lab work in Kenya.

There are also a number of people I would like to thank who contributed to the fieldwork in Ghana included in this dissertation. First, I would like to thank Philip Atiim, Mawuli Asigbee, David Sombie, and Emmaueal Osei-Mensah at Catholic Relief Services who made this work

possible by coordinating the field work with local community leaders, recruiting and helping train enumerators, and assisting in developing the study design. I would also like to thank Philip and Emmanuel for their assistance with sample collection and Emmanuel for his help coding household surveys. I would also like to acknowledge Prosper Amenuvor, Stephen Nutsugah, Roger Kanton, and Askia Mohammed at the Savanna Agricultural Research Institute for providing laboratory space and assistance that allowed us to analyze soil samples. Finally, I would like to thank fellow researchers at UIUC who contributed to this work and helped make it successful including Diana Byrne, John Trimmer and Amanda Lardizabal.

I am grateful to have had great colleagues and friends at UIUC, in the Champaign-Urbana community, and across the world, who have consistently motivated me and made me smile throughout my graduate studies. I would especially like to thank everyone in Jeremy Guest's and Helen Nguyen's research groups for thoughtfully critiquing my research presentations and answering many lab-related questions. I also thank Shaoying Qi for all of his help related to lab work. I would also like to give a huge thank you to Aaron Bryant for being a supportive friend throughout my PhD research, for watching Jasmine during my many research-related travels, and for kindly letting me to use his server to analyze my sequencing data. Finally, I would like to thank my family for being supportive of me, and for encouraging me learn and enjoy math and science at a young age, which created the foundation that led me to pursue a PhD in engineering.

I would also like to acknowledge the funding for the research in this dissertation including the National Science Foundation Graduate Research Fellowship, Carver Foundation Fellowship, CEE Distinguished Fellowship, United States Agency for International Development (USAID), and Safe Global Water Institute at the University of Illinois at Urbana-Champaign.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1 Background and Motivation

Health risks associated with fecal contamination exposure

Diarrheal disease, often caused by enteric pathogens, is the second leading cause of deaths in children under five, resulting in approximately 525,000 deaths of children under five each year.[1] Enteric pathogens are shed in human or animal feces and are transmitted through fecal-oral pathways which include water, hands, fields/floor/soil, food, and flies.[2] There are many viral, bacterial, and protozoan enteric pathogens that cause diarrhea, but a few recent large-scale studies have elucidated which pathogens have the highest attributable fraction for causing diarrhea. The Global Enteric Multicenter Study (GEMS) study analyzed over 20,000 child fecal samples from seven countries located in Africa and South Asia, and found that five pathogens (rotavirus, *Cryptosporidium* spp., *Shigella* spp., enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* producing heat stable toxin (ST-ETEC)) were responsible for the majority of moderate-to-severe diarrheal cases. Other pathogens, including *Aeromonas* spp., *Vibro cholerea* O1, and *Campylobacter jejuni*, were also found to be important diarrheal causes at specific study sites.[3] The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study was another large multisite study which included countries in South America, Africa, and Asia. This study analyzed over 30,000 child fecal samples, and found that enteric pathogens varied according to geography, but that *Campylobacter* spp., norovirus, rotavirus, astrovirus, *Shigella* spp., and *Cryptosporidium* spp. were responsible for the highest attributable fractions of diarrhea in the first or second years of life.[4] Diarrhea with blood was primarily associated with

Campylobacter spp. and *Shigella* spp., however, bloody diarrhea associated with *Shigella* spp. often had a longer duration and was more severe.[4]

In addition to the potential to cause diarrhea at infectious doses, repeated exposure to enteric pathogens may also result in environmental enteric dysfunction (EED; also referred to as environmental enteropathy). EED is a subclinical infection characterized by structural and functional changes in the gut that can lead to reduced nutrient absorption, increased intestinal permeability, and chronic gut inflammation.[5,6] The prevalence of EED is estimated to be high among people living in unsanitary conditions in low-income countries.[7] For example, a study in rural Gambia found that 95% of infants over the age of 8 months had characteristics of EED.[8] Additionally, the MAL-ED study analyzed over 24,000 non-diarrheal stool samples and found that pathogen detection was common in non-diarrheal stools,[4] which may indicate that asymptomatic enteric infections are occurring on a large scale.[9] The MAL-ED findings were also analyzed for causal pathways between enteric pathogens and EED, and *Campylobacter* spp. and *Shigella* spp. were two enteroinvasive pathogens associated with the strongest signals of gut inflammation characteristic of EED.[10]

Although there are currently no treatments available for EED, evidence exists to show that it can be reversed if individuals change to a cleaner, more sanitary environment. One study of Peace Corps volunteers published in the 1970's found that after returning back to the Unites States after living in India or Pakistan for 18- to 24-months, the small intestine of most individuals recovered functionally and morphologically to that of a healthy individual within 1 to 2 years.[11] Another study found similar recovery of small intestinal absorption and villous structure in native-born

Indians and Pakistanis after moving to New York City. However, recovery was generally less rapid for this group (taking more than 2 years for some), possibly because they had resided longer in environments with poor sanitation prior to the move.[12]

In addition to diarrhea and EED, enteric pathogen exposure and poor sanitation is also associated with increased risk of schistosomiasis,[13] trachoma,[13] and soil-transmitted helminth infections (including ascariasis/roundworm, trichuriasis/whipworm, and hookworm infections).[13,14] Soil-transmitted helminth infections have high prevalence rates low- and middle-income countries with poor sanitation, which can lead to growth faltering and anemia as well as other morbidities such as lower physical activity and poorer cognitive development.[14–16]

Repeated diarrhea episodes and EED are also associated with impaired growth,[8,17,18] which has additional negative health consequences. Stunting and other forms of undernutrition can impair a child's immune response, leading to increased risk of dying from common infectious diseases including diarrhea, pneumonia, and measles.[19–21] Undernutrition is also highly prevalent, with more than one-quarter of children under five in developing countries having linear growth deficits that classify them as stunted.[19] Due to the high prevalence and severe consequences, 14.7% of all deaths in children under five globally are attributed to stunting.[19] The structural and functional changes in the gut that occur during EED could lead to reduced child growth by decreasing the surface area in the small intestine, subsequently decreasing nutrient absorption, and by increasing the intestinal permeability and allowing more antigen molecules to penetrate the mucus layer, thereby activating the immune system and diverting energy from growth to immune response.[22] Enteric infections and stunting in childhood may also have long-term

negative consequences on health and human capital, leading to lower cognitive ability, lower adult economic productivity, and increased risk of developing obesity and associated chronic diseases later in life.[23]

Given the severity of the health and human capital consequences of enteric infections, it is critical to understand the relative importance of different transmission pathways and exposure points for enteric pathogens in low-income settings. Enteric pathogen exposure can affect child health, even when outward diarrheal symptoms are not common. However, in order for enteric pathogen exposure to be effectively reduced, a better understanding of fecal contamination sources in the domestic environment is required.

Fecal contamination in the domestic environment

Recent studies in Africa show that fecal contamination is common on household surfaces, in household and compound soils, in stored drinking water, on child and caregiver hands, and in open drainage and grey water channels.[24–29] Contamination of the domestic environment with fecal pathogens may be due to poor sanitation infrastructure or use, improper disposal of young children's feces, improper hygiene, contaminated source water, or improper management of animal feces.

Improvements in sanitation infrastructure could reduce the amount of feces entering the environment and traveling along fecal-oral transmission pathways, however, infrastructure improvements might not reduce enteric pathogens originating from young child or animal feces. Pathogens from young children's feces could still enter the environment from unhygienic

practices as children are commonly observed not to use latrines after installation[25,30,31] or to perform anal cleansing outside of a latrine,[25] and adults with access to a latrine often still unhygienically dispose of the feces from children too young to use a latrine.[32] Additionally, animal feces can contain enteric pathogens that can infect humans,[33] and animals are often kept in proximity to living quarters in low-income countries, creating an opportunity for pathogens from animal feces to be transmitted to humans through the domestic environment. Although sanitation interventions are expected to reduce fecal contamination along the transmission pathways, a recent systematic review found little to no effect of sanitation interventions on fecal contamination along the transmission pathways except for a small reduction in flies,[34] likely due to low uptake/use of sanitation interventions, as well as continued contamination from young child and animal sources.

The high prevalence of enteric infections and the complexity of transmission pathways make it critical to understand the transmission of enteric pathogens along the different pathways and the relative importance of different exposure points for enteric pathogen exposure. Hands have been shown to often be a more important exposure route than water,[27,35,36] but the relative importance of other routes of exposure is not well understood. Soil is one potentially important exposure pathway that has not been well studied and characterized, although soil ingestion by young children has been shown to be associated with increased risk of diarrhea,[37] environmental enteropathy,[38] and stunting.[38] A recent study in rural Zimbabwe found infants were observed to ingest soil during a 6-hour structured observation period, and the majority of household soil samples were contaminated with high levels of the fecal indicator bacteria *E. coli*.[28] In peri-urban Tanzania, another study found *E. coli* pathotype genes, human-specific *Bacteroidales* gene, and

enteric virus genes for enterovirus and rotavirus in soil samples from household and latrine floors.[24] The work in this dissertation integrates soil sampling into a comprehensive household sampling plan to characterize child soil ingestion activities, the potential for fecal pathogen exposure from soil ingestion, and better understand links with health.

Additionally, fomites could be an important transmission pathway of enteric pathogens in low-income countries that have not been well studied. Fomites are surfaces (porous and non-porous) and objects that become contaminated with pathogens and serve as a vehicle for disease transmission between other animate and inanimate objects.[39] Environmental contamination of fomites plays an important role in the transmission of diarrheal disease.[40] Contamination of fomites in households in low-income countries, including plates, cups, toys, food preparation surfaces, and floors are documented.[24,25,41–44] Therefore, surface cleaning may reduce disease transmission, but there have been few intervention studies to look that the effect of this on diarrheal disease transmission.[45]

Furthermore, improper disposal of child feces is common in low-income environments and can introduce fecal contamination to the environment, but the role and relative importance of this practice is not well understood. A meta-analysis of six studies found that improper handling or disposal of young children's feces was associated with a 23% increased risk of diarrhea.[46] However, all of these studies were case-control studies measuring hospital/clinic visits for diarrhea, and therefore do not provide evidence for increased risk for more severe diarrhea episodes or fecal pathogen exposure that may lead to asymptomatic enteric infection. The health risks associated with improper disposal of children's feces is important to consider because

children have higher prevalence of diarrheal disease, and therefore their feces may contain higher levels of pathogens than adults.[31] As such, exposure to children's feces is may present a greater health risk than exposure to adult feces, but the contribution of child feces to domestic fecal contamination has not been studied. To fill this critical gap, the work in this dissertation will track the contribution of young child feces separately from older child/adult feces to evaluate which one dominates fecal contamination exposure at different points throughout the transmission pathways.

In addition, animal feces can contain pathogens that can cause diarrheal disease in humans, including *Salmonella enteriditis*, *Campylobacter jejuni*, *Giardia* spp., and *Cryptosporidium parvum*.[2] Studies have found that having animals living in compounds with humans is a risk factor for diarrhea, and can be associated with an increased risk of diarrhea of more than 50%.[45] A recent systematic review found that 69% of 29 studies related to diarrhea illness and exposure to domestic livestock found a positive association between the two.[47] Associations were also found between having an animal corral in a child's sleeping area and EED and stunting among children 30 months and younger in rural Bangladesh.[48] As human feces is likely to contain more human pathogens than animal feces, it might be appropriate to prioritize proper disposal of human feces over the disposal of animal feces. However, animal feces in the household environment could cause constant exposure to enteric pathogens that may lead to EED, and more research of the link between animals and fecal contamination in the domestic environment is warranted. Recent studies have found that animal feces contamination is very common in the household environment in India and Bangladesh,[36,49,50] and that domestic animals were associated with higher levels of fecal contamination at exposure points in rural Bangladesh.[51]

Finally, when measuring enteric pathogens, it is important to understand their survival in the environment. Some human pathogens can multiply in the environment after being shed from humans or animals, increasing the risk of infection to others. This includes the enteric bacterial pathogens *E. coli*, *Shigella* spp., and some species of *Salmonella*.[2] However, this is not the case for enteric viruses, such as rotavirus, which are obligate parasites and cannot reproduce without a host. Therefore, the viability and level of infectivity of viruses on a fomite surface decreases over time.[39] The amount of time that a virus remains viable and infective on a surface is variable and complex, and influenced by a number of factors such as surface material, temperature, relative humidity, and number of other microbes present.[39,52] However, some enteric protozoa can remain viable in the environment for months.[2] A recent meta-analysis of *E. coli* survival in water and soil found that decline rates were highly variable among studies, and are not easily predictable, but that the decline rates for both commensal and pathogenic *E. coli* were significantly slower in soil compared to water.[53] The slow decline of this bacterial pathogen in soil compared to water, point to soil as being a potentially important source of exposure to bacterial pathogens in low-income settings.

Methods to identify sources of fecal contamination

Traditionally, fecal contamination is monitored using fecal indicator bacteria, such as *E. coli*, total coliforms, fecal coliforms, and enterococci,[54] however these indicators do not provide information about the actual pathogen exposure risk or the source of fecal contamination. Quantitative assessment of pathogens allows for more accurate assessment of human health risks, and this can be achieved using quantitative PCR (qPCR) to quantify target identification

genes, although this method does not provide information on the viability or infectivity of the pathogen. When using fecal indicators for point sources of pollution, these indicators generally correlate with pathogens and waterborne disease,[54] but fecal indicator bacteria are not specific to one pollution source and can originate from several human and animals fecal sources, as well as from non-fecal sources.[55] Microbial source tracking enables a more specific origin of fecal contamination to be identified, which allows the public health risk to be better characterized and pinpoints where remediation measures would be most helpful.[56] This is typically done using single-gene genetic markers specific to the feces of humans or a particular animal (e.g., ruminant, gull, canine) that can be identified in samples using PCR or quantified using qPCR,[55] and these types of markers are becoming increasingly common to assess whether fecal contamination is originating from a human or animal source in low-income countries.[24,27,36,49–51,57,58] However, not all fecal sources have a single gene marker that can be used to identify them, and this method does not allow for the identification of human feces coming from different human sources (such as young children).

Alternatively, microbial community source tracking methods rely on the diversity of microbial communities from different sources to create a microbial signature for each source that can then be used to track sources in the environment.[59] Microbial community source tracking methods can also be used to identify fecal sources of contamination that can not be identified by a specific marker gene,[60] and have previously been used to identify fecal contamination in waterways.[59–63] Since microbial communities also vary among different human fecal sources (e.g., human feces, septic tanks, sewage), community-based methods can be used for differentiating between different human sources.[64] These methods have not been previously used to discern fecal

contamination from young children. However, Yatsunenko, et al.[65] studied feces from people in Malawi, Venezuela, and the United States, and found that the bacterial diversity in a child's gut microbiome increases substantially in the first three years of life, leading to large differences (measured by UniFrac distance) between the microbial community structure in the gut microbiome of children under three years old and adults. Three years old is also the age at which some parents may begin to think it is appropriate for children to use a toilet facility themselves.[66] Therefore, we hypothesized that young children's feces (from children under three years old) can be identified and tracked separately from older child/adult feces using microbial community source tracking methods and will validate this hypothesize as part of the work in this dissertation. This is a critical step in identifying if children's feces substantially contribute to fecal contamination in the domestic environment.

1.2 Research Objectives

Current water, sanitation, and hygiene (WASH) programs in low-income countries often focus on improving water delivery and toilet/latrine infrastructure, but child exposure to fecal contamination often remains common after these types of improvements and little is known about the relative contribution of specific sources of that fecal contamination or child exposure to enteric pathogens at different points of exposure. The overall goal of this research was investigate fecal contamination and enteric pathogen transmission along several different exposure points, with a focus on young children's feces as a source of contamination and soil ingestion as an exposure point, including effects on child health. This research addresses a critical barrier to achieving maximum health gains from water, sanitation, and hygiene (WASH) investments: a lack of understanding of the role child defecation and feces disposal practices and

child soil ingestion play in the transmission of fecal-oral disease in low-income countries. The results of this research can be used to make recommendations to advance child health outcomes from WASH interventions by improving our understanding of the links between infrastructure, behavior, exposure, and health. Using evidence from this research, limited development funds for WASH could be better targeted at interventions that provide the greatest reduction of fecal pathogen exposure. More specifically, this work will identify if toilet/latrine improvement in these areas is sufficient to reduce fecal pathogen exposure or if other infrastructure and/or behavior changes are needed for a greater reduction of fecal pathogen exposure. Specific objectives of this research include:

Objective 1: To characterize the occurrence, magnitude, and distribution of enteric pathogens at different exposure points within the domestic environment of households, and assess the relationship between enteric pathogens and factors such as domestic hygiene practices and household poultry ownership.

This objective was accomplished through fieldwork in Kibera, an urban slum in Nairobi, Kenya. The high population density in urban slums can elevate the risk of disease transmission compared to rural populations. Subsequently, fecal-oral disease transmission may be at its highest within urban slum communities, and therefore our insights may have the greatest opportunity to have an impact in these contexts. Additionally, within urban areas of Sub-Saharan Africa, more than 65% of the population is estimated to live in slum communities, and the urban slum population is likely to expand due to rapid urbanization, making it a crucial population to study.[67]

Objective 2: To characterize the relative contribution of fecal contamination from young children at different potential exposure points in an urban slum environment.

To address this objective, the field study in Kibera (described in Objective 1) was leveraged. In addition to household and environmental sampling, microbial community methods for source tracking young child fecal sources separately other human-associated fecal sources were also developed and validated.

Objective 3: To evaluate the relationship between child feces disposal practices and child growth in low- and middle-income countries.

To accomplish this objective, Demographic and Health Surveys (DHS) data for 34 low- and middle-income countries was used to evaluate the association between child feces disposal practices and child stunting (as well as other measures of child growth). This work also evaluated if additional child health benefits could be achieved from caregivers engaging in more hygienic child feces disposal behaviors among households that already had access to toilet facilities.

Objective 4: To characterize the prevalence of soil ingestion in young children and evaluate the relationship between child soil ingestion and diarrhea.

As part of the work to accomplish this objective, it was also assessed if household flooring in the bedroom (earth versus finished) affected the prevalence, frequency, or amount of soil ingested, what the frequency of soil ingestion was in children over the course of a week, and how caregivers perceived the act of their children ingesting soil. This objective was pursued both in an urban slum (Kibera) and a rural setting in Northern Ghana.

1.3 Dissertation Organization

This dissertation is organized into the following chapters:

Chapter 2: Enteric Pathogens from Water, Hands, Surface, Soil, Drainage Ditch, and Stream Exposures Points in a Low-income Neighborhood of Nairobi, Kenya

In this chapter, results are presented for the occurrence, magnitude, and distribution of fecal contamination and four enteric pathogens at several exposure points inside and outside of households in the Kibera urban slum neighborhood in Nairobi, Kenya. The relationships between fecal pathogen detection and hygiene practices (e.g., handwashing, surface cleaning) and other household characteristics (e.g., poultry ownership) were also evaluated. Finally, the interactions among different pathways for pathogen transmission and the ability of the fecal indicator *E. coli* to estimate pathogen contamination were also assessed in this chapter. Widespread enteric pathogen contamination was found inside and outside households, despite every household having access to a toilet, prompting the investigation into specific sources of the widespread contamination in this environment, detailed in Chapter 3. High levels of pathogen contamination was also detected in soils, motivating further work to better characterize soil ingestion by children and potential health implications, detailed in Chapters 5 and 6.

Chapter 3: Microbial Source Tracking of Young Children's Feces: Evaluation of Methods and Environmental Fecal Contamination in Kenya

In this chapter, the use of microbial community source tracking methods is proposed to track young children's feces separately from older children and adult feces. These methods are evaluated using two different primer sets and several different analysis techniques on spiked

water and soil samples, and method recommendations are given based on techniques that provided high levels of sensitivity and specificity for identifying the dominant source of contamination in samples as well as correctly identifying the presence and absence of a contamination source. The newly developed source tracking methods are then used to separately track the contribution of young child feces and older child/adult feces among human fecal contamination in environmental samples that were collected in Kibera as part of the study introduced in Chapter 2. Among samples contaminated with human fecal contamination, feces from young children was found to be dominant in samples taken from the indoor environment (caregiver and child hands, tables, plates), feces from older children and adults tended to be dominant in samples taken from standing water and streams in the outdoor environment, and both were dominant in open drainage ditch samples. The finding that child feces dominated human fecal contamination present inside households motivated the evaluation of the effects of child feces disposal practices on child health on a large scale, detailed in Chapter 4.

Chapter 4: The Effect of Young Children's Feces Disposal Practices on Child Growth: Evidence from 34 Countries

In this chapter, nationally representative survey data from 34 low- and middle-income countries was used to evaluate the association between child feces disposal practices and stunting, as well as other anthropometric measures of child growth and nutritional status. The relationship between child feces disposal and child growth was evaluated at the household level for improved disposal (disposal in an improved toilet) and unimproved disposal (disposal in an unimproved toilet), as well as at the community-coverage level for improved disposal.

Chapter 5: Soil Ingestion Is Associated with Child Diarrhea in an Urban Slum of Nairobi, Kenya

In this chapter, survey data from the study in Kibera (introduced in Chapter 2) was used to assess the prevalence of soil ingestion among children aged 3 months to 5 years and to evaluate the relationship between soil ingestion and diarrhea, which is the first study to evaluate this relationship in households with primarily finished floors. Soil samples were also analyzed for fecal contamination and a human-associated marker, and found to have high levels of contamination.

Chapter 6: Child Soil Ingestion: Frequency, Relationship with Household Floor Material, Caregiver Perceptions, and Associations with Child Diarrhea in Rural Ghana

In this chapter, we use survey data collected for over 500 children under five in rural Ghana to characterize the prevalence, frequency, and quantity of soil ingestion. The relationship between soil ingestion and household floor material (earth vs. finished) was also evaluated, as was the relationship between soil ingestion and diarrhea. Finally, the relationship between caregiver perceptions of soil ingestion were assessed against whether or not a caregiver reported stopping their child from ingesting soil, and the quantity of soil consumed.

Chapter 7: Conclusions

This chapter provides a summary of the work presented in previous chapters, as well as a summary of conclusions and contributions. Future research directions to build on the research presented in this dissertation are also presented.

1.4 References

1. World Health Organization. Diarrhoeal disease fact sheet. *WHO* (2017). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/. (Accessed: 6th June 2017)
2. Curtis, V., Cairncross, S. & Yonli, R. Review: Domestic hygiene and diarrhoea–pinpointing the problem. *Trop Med Int Health* 5, 26–30 (2000).
3. Kotloff, K. L. *et al.* Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet* 382, 209–222 (2013).
4. Platts-Mills, J. A. *et al.* Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob. Health* (2015).
5. Humphrey, J. H. Child undernutrition, tropical enteropathy, toilets, and handwashing. *The Lancet* 374, 1032–1035 (2009).
6. Korpe, P. S. & Petri, W. A. Environmental enteropathy: critical implications of a poorly understood condition. *Trends Mol. Med.* 18, 328–336 (2012).
7. Prendergast, A. & Kelly, P. Enteropathies in the Developing World: Neglected Effects on Global Health. *Am. J. Trop. Med. Hyg.* 86, 756–763 (2012).
8. Lunn, P. G. The impact of infection and nutrition on gut function and growth in childhood. *Proc. Nutr. Soc.* 59, 147–154 (2000).
9. Gilmartin, A. A. & Petri, W. A. Exploring the role of environmental enteropathy in malnutrition, infant development and oral vaccine response. *Philos. Trans. R. Soc. B Biol. Sci.* 370, 20140143–20140143 (2015).
10. Kosek, M. N., Investigators, M.-E. N. & others. Causal Pathways from Enteropathogens to Environmental Enteropathy: Findings from the MAL-ED Birth Cohort Study. *EBioMedicine* 18, 109–117 (2017).
11. Lindenbaum, J., Gerson, C. D. & Kent, T. H. Recovery of small-intestinal structure and function after residence in the tropics. I. Studies in Peace Corps volunteers. *Ann. Intern. Med.* 74, 218–222 (1971).
12. GERSON, C. D., Kent, T. H., SAHA, J. R., Siddiqi, N. & Lindenbaum, J. Recovery of Small-Intestinal Structure and Function After Residence in the TropicsII. Studies in Indians and Pakistanis Living in New York City. *Ann. Intern. Med.* 75, 41–48 (1971).
13. Esrey, S. A., Potash, J. B., Roberts, L. & Shiff, C. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. *Bull. World Health Organ.* 69, 609 (1991).
14. Ziegelbauer, K. *et al.* Effect of Sanitation on Soil-Transmitted Helminth Infection: Systematic Review and Meta-Analysis. *PLoS Med.* 9, e1001162 (2012).
15. Bethony, J. *et al.* Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet* 367, 1521–1532 (2006).
16. Mara, D., Lane, J., Scott, B. & Trouba, D. Sanitation and Health. *PLoS Med.* 7, e1000363 (2010).
17. Mbuya, M. N. & Humphrey, J. H. Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries. *Matern. Child. Nutr.* 12, 106–120 (2016).
18. Checkley, W. *et al.* Multi-country analysis of the effects of diarrhoea on childhood stunting. *Int. J. Epidemiol.* 37, 816–830 (2008).

19. Black, R. E. *et al.* Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet* 382, 427–451 (2013).
20. Olofin, I. *et al.* Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: a pooled analysis of ten prospective studies. *PloS One* 8, e64636 (2013).
21. Caulfield, L. E., de Onis, M., Blössner, M. & Black, R. E. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am. J. Clin. Nutr.* 80, 193–198 (2004).
22. Arnold, B. F. *et al.* Cluster-randomised controlled trials of individual and combined water, sanitation, hygiene and nutritional interventions in rural Bangladesh and Kenya: the WASH Benefits study design and rationale. *BMJ Open* 3, e003476 (2013).
23. Guerrant, R. L., DeBoer, M. D., Moore, S. R., Scharf, R. J. & Lima, A. A. M. The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease. *Nat. Rev. Gastroenterol. Hepatol.* 10, 220–229 (2012).
24. Pickering, A. J. *et al.* Fecal Contamination and Diarrheal Pathogens on Surfaces and in Soils among Tanzanian Households with and without Improved Sanitation. *Environ. Sci. Technol.* 46, 5736–5743 (2012).
25. Exley, J. L. R., Liseka, B., Cumming, O. & Ensink, J. H. J. The Sanitation Ladder, What Constitutes an Improved Form of Sanitation? *Environ. Sci. Technol.* 49, 1086–1094 (2015).
26. Katukiza, A. Y., Ronteltap, M., van der Steen, P., Foppen, J. W. A. & Lens, P. N. L. Quantification of microbial risks to human health caused by waterborne viruses and bacteria in an urban slum. *J. Appl. Microbiol.* 116, 447–463 (2014).
27. Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J. & Boehm, A. B. Hands and Water as Vectors of Diarrheal Pathogens in Bagamoyo, Tanzania. *Environ. Sci. Technol.* 47, 355–363 (2013).
28. Ngure, F. M. *et al.* Formative Research on Hygiene Behaviors and Geophagy among Infants and Young Children and Implications of Exposure to Fecal Bacteria. *Am. J. Trop. Med. Hyg.* 89, 709–716 (2013).
29. Pickering, A. J. *et al.* Hands, Water, and Health: Fecal Contamination in Tanzanian Communities with Improved, Non-Networked Water Supplies. *Environ. Sci. Technol.* 44, 3267–3272 (2010).
30. Clasen, T. F. *et al.* Interventions to improve disposal of human excreta for preventing diarrhoea. in *Cochrane Database of Systematic Reviews* (ed. The Cochrane Collaboration) (John Wiley & Sons, Ltd, 2010).
31. Brown, J., Cairncross, S. & Ensink, J. H. J. Water, sanitation, hygiene and enteric infections in children. *Arch. Dis. Child.* 98, 629–634 (2013).
32. Majorin, F. *et al.* Child Feces Disposal Practices in Rural Orissa: A Cross Sectional Study. *PLoS ONE* 9, e89551 (2014).
33. Vasco, K., Graham, J. P. & Trueba, G. Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in ecuador. *Appl. Environ. Microbiol.* 82, 4218–4224 (2016).
34. Sclar, G. D. *et al.* Assessing the impact of sanitation on indicators of fecal exposure along principal transmission pathways: A systematic review. *Int. J. Hyg. Environ. Health* 219, 709–723 (2016).

35. Mattioli, M. C. M., Davis, J. & Boehm, A. B. Hand-to-Mouth Contacts Result in Greater Ingestion of Feces than Dietary Water Consumption in Tanzania: A Quantitative Fecal Exposure Assessment Model. *Environ. Sci. Technol.* 49, 1912–1920 (2015).
36. Schriewer, A. *et al.* Human and Animal Fecal Contamination of Community Water Sources, Stored Drinking Water and Hands in Rural India Measured with Validated Microbial Source Tracking Assays. *Am. J. Trop. Med. Hyg.* 14–0824 (2015). doi:10.4269/ajtmh.14-0824
37. Shivoga, W. A. & Moturi, W. N. Geophagia as a risk factor for diarrhoea. *J. Infect. Dev. Ctries.* 3, 94–98 (2009).
38. George, C. M. *et al.* Geophagy is associated with environmental enteropathy and stunting in children in rural Bangladesh. *Am. J. Trop. Med. Hyg.* 92, 1117–1124 (2015).
39. Boone, S. A. & Gerba, C. P. Significance of Fomites in the Spread of Respiratory and Enteric Viral Disease. *Appl. Environ. Microbiol.* 73, 1687–1696 (2007).
40. Van, R., Marrow, A. L., Reves, R. R. & Pickering, L. K. Environmental contamination in child day-care centers. *Am. J. Epidemiol.* 133, 460–470 (1991).
41. Julian, T. R. *et al.* Fecal Indicator Bacteria Contamination of Fomites and Household Demand for Surface Disinfection Products: A Case Study from Peru. *Am. J. Trop. Med. Hyg.* 89, 869–872 (2013).
42. Stauber, C., Walters, A., de Aceituno, A. & Sobsey, M. Bacterial Contamination on Household Toys and Association with Water, Sanitation and Hygiene Conditions in Honduras. *Int. J. Environ. Res. Public. Health* 10, 1586–1597 (2013).
43. Vujcic, J. *et al.* Toys and toilets: cross-sectional study using children's toys to evaluate environmental faecal contamination in rural Bangladeshi households with different sanitation facilities and practices. *Trop. Med. Int. Health* 19, 528–536 (2014).
44. Exum, N. G. *et al.* Floors and toilets: association of floors and sanitation practices with fecal contamination in Peruvian Amazon peri-urban households. *Environ. Sci. Technol.* 50, 7373–7381 (2016).
45. Curtis, V. *et al.* Hygiene: new hopes, new horizons. *Lancet Infect. Dis.* 11, 312–321 (2011).
46. Gil, A., Lanata, C., Kleinau, E. & Penny, M. Strategic Report 11 Children's Feces Disposal Practices in Developing Countries and Interventions to Prevent Diarrheal Diseases. *US Agency Int. Dev. Wash. DC* 20523, (2004).
47. Zambrano, L. D., Levy, K., Menezes, N. P. & Freeman, M. C. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. *Trans. R. Soc. Trop. Med. Hyg.* 108, 313–325 (2014).
48. George, C. M. *et al.* Fecal markers of environmental enteropathy are associated with animal exposure and caregiver hygiene in Bangladesh. *Am. J. Trop. Med. Hyg.* 93, 269–275 (2015).
49. Harris, A. R. *et al.* Ruminants contribute fecal contamination to the urban household environment in Dhaka, Bangladesh. *Environ. Sci. Technol.* 50, 4642–4649 (2016).
50. Boehm, A. B. *et al.* Occurrence of host-associated fecal markers on child hands, household soil, and drinking water in rural Bangladeshi households. *Environ. Sci. Technol. Lett.* 3, 393–398 (2016).
51. Ercumen, A. *et al.* Animal feces contribute to domestic fecal contamination: Evidence from E. coli measured in water, hands, food, flies and soil in Bangladesh. *Environ. Sci. Technol.* (2017).
52. Kim, S. J., Si, J., Lee, J. E. & Ko, G. Temperature and Humidity Influences on Inactivation Kinetics of Enteric Viruses on Surfaces. *Environ. Sci. Technol.* 46, 13303–13310 (2012).

53. Franz, E., Schijven, J., de Roda Husman, A. M. & Blaak, H. Meta-Regression Analysis of Commensal and Pathogenic *Escherichia coli* Survival in Soil and Water. *Environ. Sci. Technol.* 140602063414009 (2014). doi:10.1021/es501677c
54. *Microbial Source Tracking: Methods, Applications, and Case Studies*. (Springer New York, 2011).
55. Boehm, A. B. *et al.* Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. *Water Res.* 47, 6812–6828 (2013).
56. Reischer, G. H. *et al.* Performance Characteristics of qPCR Assays Targeting Human- and Ruminant-Associated *Bacteroidetes* for Microbial Source Tracking across Sixteen Countries on Six Continents. *Environ. Sci. Technol.* 47, 8548–8556 (2013).
57. Jenkins, M. W., Tiwari, S., Lorente, M., Gichaba, C. M. & Wuertz, S. Identifying human and livestock sources of fecal contamination in Kenya with host-specific Bacteroidales assays. *Water Res.* 43, 4956–4966 (2009).
58. Mattioli, M. C. *et al.* Enteric Pathogens in Stored Drinking Water and on Caregiver's Hands in Tanzanian Households with and without Reported Cases of Child Diarrhea. *PLoS ONE* 9, e84939 (2014).
59. Henry, R. *et al.* Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters. *Water Res.* 93, 242–253 (2016).
60. Unno, T. *et al.* Use of Barcoded Pyrosequencing and Shared OTUs To Determine Sources of Fecal Bacteria in Watersheds. *Environ. Sci. Technol.* 44, 7777–7782 (2010).
61. Cao, Y., Van De Werfhorst, L. C., Sercu, B., Murray, J. L. S. & Holden, P. A. Application of an Integrated Community Analysis Approach for Microbial Source Tracking in a Coastal Creek. *Environ. Sci. Technol.* 45, 7195–7201 (2011).
62. Newton, R. J., Bootsma, M. J., Morrison, H. G., Sogin, M. L. & McLellan, S. L. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. *Microb. Ecol.* 65, 1011–1023 (2013).
63. Dubinsky, E. A. *et al.* Application of Phylogenetic Microarray Analysis to Discriminate Sources of Fecal Pollution. *Environ. Sci. Technol.* 46, 4340–4347 (2012).
64. Cao, Y. *et al.* Evaluation of molecular community analysis methods for discerning fecal sources and human waste. *Water Res.* 47, 6862–6872 (2013).
65. Yatsunenko, T. *et al.* Human gut microbiome viewed across age and geography. *Nature* (2012). doi:10.1038/nature11053
66. Yeager, B. A., Huttly, S. R., Bartolini, R., Rojas, M. & Lanata, C. F. Defecation practices of young children in a Peruvian shanty town. *Soc. Sci. Med.* 49, 531–541 (1999).
67. Brown, J. *et al.* A controlled, before-and-after trial of an urban sanitation intervention to reduce enteric infections in children: research protocol for the Maputo Sanitation (MapSan) study, Mozambique. *BMJ Open* 5, (2015).

CHAPTER 2: ENTERIC PATHOGENS FROM WATER, HANDS, SURFACE, SOIL, DRAINAGE DITCH, AND STREAM EXPOSURE POINTS IN A LOW-INCOME NEIGHBORHOOD OF NAIROBI, KENYA*

2.1 Abstract

Child exposure to fecal-oral pathogens occurs through several transmission pathways, however, the relative importance of different exposure points for pathogen transmission is not well understood. We conducted a cross-sectional study in the urban slum of Kibera in Nairobi, Kenya, collecting environmental samples from source water, stored drinking water, caregiver hands, child hands, household surfaces, soil, standing water, open drainage ditches, and streams from 40 households. We enumerated *Escherichia coli* colony forming units (CFU), and quantified gene copies for the following enteric pathogens: Adenovirus, *Campylobacter jejuni*, *Shigella* spp./enteroinvasive E. coli (EIEC), and *Vibrio cholerae*. At least one of these pathogens was detected in 13% of household stored water, 47% of hand, 46% of table surface, 26% of plate surface, 75% of floor surface, 96% of soil, 56% of standing water, 77% of drainage ditch, and 100% of stream samples despite all households having access to a toilet or latrine. Our results provide evidence that children are exposed to enteric pathogens from several exposure points at the same time, that there are interactions between different transmission pathways, that ownership of chickens in this urban setting was associated with increased detection of *C. jejuni* inside households and on soil, and that *V. cholerae* was detected at several exposure points during a cholera outbreak.

* This chapter is in preparation: Bauza, V., Ocharo, R., Madadi, V., Nguyen, T.H., Guest, J.S. Enteric pathogens from water, hands, surface, soil, drainage ditch, and stream exposures points in a low-income neighborhood of Nairobi, Kenya. To be submitted to *Environmental Science and Technology.*

2.2 Introduction

Exposure to enteric pathogens can cause diarrhea, which is the second leading cause of death in children under five.[1] Repeated exposure to enteric pathogens may also cause environmental enteric dysfunction (EED; as referred to as environmental enteropathy) with characteristic structural and functional changes in the gut that lead to reduced nutrient absorption, increased intestinal permeability, and chronic gut inflammation in the absence of diarrhea,[2,3] which is believed to be widespread among children in low-income countries.[4,5] Repeated diarrheal episodes and EED have been associated with impaired growth,[4,6,7] which can increase the risk of death[1,6,8] and lead to reductions in human capital including lower cognitive ability and adult economic productivity.[9] Given the severity of the health and human capital consequences of enteric infections, it is critical to understand the transmission pathways and exposure points for fecal pathogens in low-income settings.

There are several viral, bacterial, and protozoan enteric pathogens that are known to cause diarrhea, but a few recent multi-site studies have shed light on which pathogens cause the highest attributable fraction of diarrhea cases. The Global Enteric Multicenter Study (GEMS) study, which analyzed over 20,000 child fecal samples from seven countries in Africa and South Asia, found that rotavirus, *Cryptosporidium* spp., *Shigella* spp., enteropathogenic *Escherichia coli* (EPEC), and enterotoxigenic *E. coli* producing heat stable toxin (ST-ETEC) were the pathogens responsible for the majority of moderate-to-severe diarrheal cases in Africa and Asia (with pathogens such as *Vibro cholerea* O1 and *Campylobacter jejuni* important diarrheal causes in specific areas).[10] Another large multisite study in South America, Africa, and Asia (referred to as the MAL-ED study) analyzed over 30,000 child fecal samples. While there was substantial

variation in fecal pathogens according to geography, *Camplyobacter* spp, norovirus GII, rotavirus, astrovirus, *Shigella* spp.*,* and *Cryptosporidium* spp. were responsible for the highest attributable fractions to diarrhea for the first or second years of life.[11] Additionally, infection with multiple pathogens was associated with diarrhea and the odds of diarrhea increased with each additional co-infecting pathogen.[11] Enteroinvasive pathogens such as *Campylobacter* spp.*, Shigella* spp.*,* and enteroinvasive *E. coli* (*EIEC)* were also associated with strong signals of gut inflammation characteristic of EED.[12] The results of these studies demonstrate multiple enteric pathogens contribute to the child disease burden, but certain pathogens have a greater contribution to this burden. This makes it important to understand and assess the potential for exposure to multiple enteric pathogens, particularly pathogens that have been identified as important causes of diarrhea and pathogens identified to contribute to gut inflammation characteristic of EED.

Enteric pathogens can originate from human or animal feces and are transmitted through fecal-oral pathways including water, fields/floor, hands, flies, and food.[13] Improvements in sanitation infrastructure such as latrine installation could reduce the amount of feces entering the environment and traveling along these pathways, however, these measures may not reduce enteric pathogens originating from young child or animal feces. Pathogens could still enter the environment from unhygienic practices as children are commonly observed to perform anal cleansing outside of the latrine[14] or to not use latrines after installation,[14–16] and it is not uncommon for adults with access to a latrine to unhygienically dispose of the feces from children too young to use a latrine.[17,18] Additionally, animal feces can contain enteric pathogens such as *Camplylobacter jejuni* that can infect humans,[19] and animals are often kept close to living

quarters in low-income countries, providing an opportunity for pathogens from animal feces to enter and spread in the environment. The high prevalence of enteric infections and the complexity of transmission pathways makes it critical to understand the transmission of enteric pathogens along the different pathways, however, most work has focused on measuring fecal indicator bacteria, which can also have non-fecal sources[20] and do not always correlate well with pathogens.[21,22] Taken together, these observations indicate that enteric pathogen transmission can still be important in low-income settings with access to sanitation facilities, but there is limited knowledge of enteric pathogen transmission along multiple routes or how the transmission routes interact for specific pathogens, particularly in low-income urban settings.

The overarching goal of this work is to advance our understanding of the distribution of enteric pathogens along different transmission pathways within the domestic environment. In pursuit of this goal, specific objectives were: (i) to characterize pathogen prevalence at multiple exposure points along transmission pathways, (ii) to evaluate the relationship of fecal pathogens with hygiene practices and other household characteristics (e.g., poultry ownership), (iii) to evaluate associations and potential interactions of enteric pathogens between different exposure points and transmission pathways, and (iv) to assess the efficacy of the fecal indicator *E. coli* as a surrogate for pathogen contamination. Household interviews were conducted and environmental samples were collected from 40 households in the low-income urban neighborhood of Kibera in Nairobi, Kenya. Environmental samples were collected from source water, stored drinking water, caregiver and child hands, household surfaces (table, plate, floor), soil, open drainage ditches, standing water, and streams. Samples were analyzed for *E. coli* and for genes from the enteric pathogens human adenovirus (hexon), *C. jejuni* (ciaB), *Shigella* spp./EIEC (ipaH), and *Vibrio*

cholerae (ctxA). These data were leveraged to advance our understanding of the distribution of fecal pathogens within the domestic environment to inform and prioritize future WASH interventions and to better elucidate links between poor sanitation and hygiene practices, fecal pathogen exposure, and negative child health outcomes.

2.3 Methods and Materials

Study site and household selection

This study was conducted in the Kibera urban slum in Nairobi, Kenya in June 2015. Households in the study site were clustered into compounds that shared outdoor open space and usually shared the same toilet/latrine and water source. Compounds were purposively selected for inclusion in this study from three separate wards (Makina, Sarangombe, and Lindi) in Kibera, in an attempt to increase the diversity of sanitation and drainage infrastructure serving included compounds. Households with children under five in each selected compound were randomly selected for inclusion, with the requirement that at least one household included had to have a child under three for each compound. Once informed consent was completed, household interviews were conducted with the primary caregiver to obtain information about household demographics and water, sanitation, and hygiene behaviors. 55 children under five years old were included in this study, from a total of 40 households and 16 compounds. This study was conducted using the same households as Bauza et al.,[23] which evaluated the association between child soil ingestion and diarrhea, as well as soil contamination with *E. coli* and a human-associated fecal marker. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign and the National Commission for Science, Technology and Innovation in Kenya.

Environmental sample collection

A total of 237 environmental samples were collected from source water, stored drinking water, caregiver and child hands, household surfaces (table, plate, floor), soil, open drainage ditches, standing water, and streams.

Water sampling. Source water and stored household drinking water samples were collected from each household using sterile Whirl-Pak bags. For stored household water samples, the respondent was asked to retrieve water the way she would for a child and pour the water into the bag. The enumerator asked if anything had been done to treat the water, and if so, how long ago the water had been treated. Water was tested for free and total chlorine content prior to sample collection using a chlorine test strip (Hach, Loveland, CO), and water samples that tested positive for chlorine were collected in sterile Whirl-Pak bags containing sodium thiosulfate to neutralize the chlorine.

Hand rinse sampling. Hand rinse samples were collected from one caregiver and one child at each household. Respondents were asked to place their hands, one at a time, into a 24-oz sterile Whirl-Pak bag containing 150 mL of sterile phosphate-buffered saline (PBS). Each hand was massaged through the bag for 30 seconds. The respondent was asked how much time had past since she washed her hands last and since her child's hands had last been washed.

Soil sampling. Where applicable, soil samples were collected from the household entrance and/or shared common space near each household. Details of soil sample collection and processing are described in Bauza et al.[23]

Surface sampling. Surface samples were collected from two of the following three items in each household: a table, a plate or bowl, and the floor. Nylon-flocked swabs in liquid Amies elution solution (Eswab, BD, Franklin Lakes, NJ) were used to sample surfaces. Duplicate swab samples were collected at each location. For sampling, the wetted tip of the swab was used to wipe a 10 cm by 10 cm surface, wiping in the horizontal direction with one side of the swab and the vertical direction with the other side of the swab. The swab was placed back in a sterile tube containing 1 mL of Amies elution solution until analysis. At the time of sampling, the surface material, time since last cleaning of surface, temperature, and relative humidity were recorded.

Open drainage ditch and standing water sampling. Any open drainage ditches or large standing water puddles within the compound (or immediately adjacent) were sampled. Sterile polystyrene sampling spoons were used for sample collection and transfer to 15 mL sterile centrifuge tubes (Corning Inc., Corning, NY).

Stream sampling. Samples from streams near compounds were collected by dipping a Whirl-Pak bag into the stream at an access point near the compound. Bags were filled with approximately 500 ml of stream water.

E. coli enumeration

E. coli was enumerated by using m-ColiBlue24 Broth media (Hach, Loveland, CO) following the manufacturer's protocol approved by the US EPA. Plates were incubated at 35°C for 24 hours. 100 mL of each water sample and 10 mL of each hand rinse sample was filtered individually through 47 mm, 0.45 µm pore size mixed cellulose esters filters (Pall Corporation, Port Washington, NY). For soil samples, bacteria were eluted in PBS from 2 g of soil, diluted, and filtered. For surface swab samples, swabs were vortexed in the tube for 20 sec and removed from the tube while pressing the swab against the sides of the tube to recover the liquid in the swab, and the 1 mL of wetting solution was filtered and the tube was rinsed with PBS that was also filtered. For open drainage ditch and standing water samples, a 10^{-3} dilution was filtered. Stream samples were filtered at a dilution ranging from no dilution to 10^{-6}, with the level of sample dilution increasing over time due to previous dilutions exceeding the quantification limit. All samples were transported and stored in a cooler on ice until they were processed within 8 hours of collection. The limit of detection (LOD) and upper limit of quantification (LLOQ) varied by sample type due to differential sampling procedures, and each are reported in SI.

DNA extraction

DNA was extracted from a 0.25 g sample for soil samples, 1 mL of wetting solution for swab samples, 1 mL of open drainage ditch and standing water samples within eight hours of collection using PowerSoil DNA Isolation kit (MO BIO Laboratories, Inc., Carisbad, CA) following manufacturer's guidelines.

For water, hand rinse, and stream samples, 250 mL of each water sample, 50 mL of each hand rinse sample, and 1-10 mL of each stream sample was filtered separately through 47 mm, 0.45 μm pore size mixed cellulose esters HA filters (Millipore, Billerica, MA) for downstream molecular processing. Filters were aseptically rolled into 5 mL transport tubes (Eppendorf, Hamburg, Germany) and frozen at -20°C for up to 3 weeks, transported back to the University of Illinois at Urbana-Champaign in a cooler with ice packs, and stored at -80°C until DNA extraction. DNA was extracted from water and hand rinse filters using PowerWater DNA Isolation kit (MO BIO Laboratories, Inc., Carisbad, CA) following manufacturer's guidelines.

Pathogen assays

Human adenovirus, *Campylobacter jejuni*, *Shigella* spp./EIEC, and *Vibrio cholerae* were analyzed via quantitative PCR (qPCR) for all samples that were positive for *E. coli*. Adenovirus, a double-stranded DNA virus, was selected as an indicator of viral enteric pathogens because RNA viruses such as rotavirus would have been more likely to degrade during transport to the US after extraction in Kenya. *C. jejuni* and *Shigella* spp. were selected for their links to moderate-to-severe (including bloody) diarrhea and gut inflammation characteristic of EED. *C. jejnui* was also selected because of its potential zoonotic transmission from poultry. *V. cholerae* was selected because there was a cholera outbreak in the study area at the time of the study.

The target gene used to detect adenovirus was the hexon, *C. jejuni* was *ciaB*, *Shigella* spp./EIEC was *ipaH*, and *V. cholerae* was *ctxA*. Previously published primers and hydrolysis probes were used for each assay (Table A.1 of Supporting Information [SI]).[24–26] 15 μL qPCR reactions were created for each assay on a 384-well plate, including 2 μL of sample DNA. Master mix

contained 1X final concentration of TaqMan™ Universal PCR Master Mix (Applied Biosystems, Waltham, MA), 500 nM of each primer, 100 nM of probe, and 20 ng/µL Bovine Serum Albumin (BSA; Invitrogen, Waltham, MA). Samples were amplified on an Applied Biosystems 7900HT Fast Real-Time PCR System using thermocycle conditions of 2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 sec at 95°C and 1 min at 60°C (with the exception of *V. cholerae*, for which 40 cycles of the same conditions were used). Standard curves were generated using 10-fold serial dilutions for standard concentrations of 3 to 3×10^6 genes copies·μL^{-1}. Plasmid standards were used for the *C. jejuni*, *Shigella* spp./EIEC, and *V. cholerae* reactions, and a synthesized DNA standard was used for the adenovirus reaction (Table A.2 of SI). Standard dilutions were run in triplicate on each plate, at least 10% of environmental samples for each sample type were run in duplicate, and each plate also included three no-template control samples. The linearity and efficiency of all standard curves were within acceptable ranges with linearity greater than 0.99 and efficiency ranging from 94%-107% (specific values reported in Section A.1, SI). PCR inhibition was evaluated in a subset of at least 10% of samples from each sample type using a linearity of response method similar to the method recommended by Cao et al[27] (method details and detailed inhibition results reported in Section A.1, SI). The majority of samples showed no signs of inhibition, although minor inhibition was detected in multiple soil samples.

The LOD and lower limit of quantification (LLOQ) varied by sample type due to differential sampling procedures (each are reported in Table A.3, SI). The LOD was calculated by assuming a theoretical minimum detection of 3 copies per PCR reaction, as recommended by Bustin et al.[28] The LLOQ was the lowest concentration with at least 50% of the standard curve replicates

amplified, which was 3 copies·μL^{-1} for all assays. Samples were quantifiable if their quantification cycle threshold (C_q) values were equal to or above the LLOQ. Samples with amplification detected that were below the LLOQ were considered detected but not quantified (DNQ), and were classified as positive for the target gene.

Statistical analysis

Stata version 13.1 (StataCorp LP, College Station, TX) was used for statistical analyses. T-tests and Fisher's exact tests were used to test for differences in *E. coli* and pathogen detection and concentration among variables, such as domestic hygiene practices. Fisher's exact tests and t-test were used to test for associations between pathogens detected at different exposure points. Pearson's correlation coefficients were also calculated to test the linearity of the correlation between count data for two different exposure points in order to assess possible interactions among pathways as well as the potential for one sampling location to serve as a surrogate for another sampling location (for example, to assess if caregiver hands could represent pathogen concentration on child hands in a future study).

E. coli CFU and pathogen gene copy count data was log transformed to normalize their distributions prior to statistical tests. For quantitative analysis involving *E. coli*, half the LOD value was assigned to negative samples and 550 CFU/filter was assigned to samples that were too numerous to count (>500 CFU/filter). For quantitative analysis involving pathogens, the LOD was assigned as a value to DNQ samples and half the LOD was assigned as a value to samples that did not detect the target gene.

2.4 Results and Discussion

Household characteristics

All households included in this study had access to a toilet facility and reported that all adults in the household used the facility. 95% of households used a shared toilet facility, and 17 households (42.5%) used a pour flush toilet with a sewer connection, 19 households (47.5%) used a pit latrine with slab, 3 households (7.5%) used a pit latrine without slab, and 1 household (2.5%) used a pour flush pit latrine. Additionally, the feces from 40% of children under five was disposed of in a toilet/latrine, with the remaining feces from children being disposed of in a ditch (24%), river or stream (16%), garbage (15%) or left in the open (4%). Five households (12.5%) reported owning livestock, and chickens were visible in the household or compound area for each of these households. No other livestock was observed in or near compounds included in our study, although dogs were observed near some of the included compounds.

Prevalence of *E. coli* and enteric pathogens

There was a high prevalence of *E. coli*, adenovirus, *Campylobacter jejuni*, *Shigella* spp./EIEC, and *Vibrio cholerae* at multiple exposure locations (Figure 2.1). *E. coli* was detected at a higher rate than pathogens, 20% in of source water, 63% of stored drinking water, 98% of caregiver hand, 87% of child hand, 89% of table surface, 65% of plate surface, 100% of floor, soil, standing water, drainage ditch, and stream samples. At least one enteric pathogen was detected in 13% of stored drinking water, 54% of caregiver hand, 37% of child hand, 46% of table surface, 26% of plate surface, 75% of floor surface, 96% of soil, 56% of standing water, 77% of drainage ditch, and 100% of stream samples (means and standard deviation of quantified pathogen counts for each sample type are provided in Table A.4 in the SI). Of the enteric pathogens assayed,

Adenovirus was most commonly detected inside households followed by *V. cholerae*, *C. jejuni*, and *Shigella* spp./EIEC. Outside households *Shigella* spp./EIEC was most commonly detected, followed by *C. jejuni*, Adenovirus, and *V. cholerae*. Additionally, the most commonly detected pathogen in household water was *V. cholerae*, on hands was Adenovirus, on tables and plates was *V. cholerae*, on floors was *C. jejuni*, in soil was *Shigella* spp./EIEC, in standing water and drains were *C. jejuni* and *Shigella* spp./EIEC, and in streams were Adenovirus and *Shigella* spp./EIEC. This variation illustrates that different pathogens were more frequently detected at different exposure points, which could influence the type of intervention that could be most effective at reducing exposure to pathogens.

Taking a closer look at which pathogens were most common at individual exposure points, *V. cholerae* was the least detected pathogen in soil and was detected at a lower frequency in soil than *C. jejuni* ($p<0.0001$), *Shigella* spp./EIEC ($p<0.0001$), and Adenovirus ($p=0.0267$). *C. jejuni* ($p=0.0056$) and *Shigella* spp./EIEC ($p<0.0001$) were also detected at a higher frequency compared to adenovirus in soil, but there was no difference between the detection frequencies of *C. jejuni* and *Shigella* spp./EIEC ($p=0.263$). *V. cholerae* was more likely to be detected in water samples than Adenovirus ($p=0.0429$) or *Shigella* spp./EIEC ($p=0.0429$). In drainage ditch samples, *Shigella* spp./EIEC was more likely to be positive than *V. cholerae* ($p=0.0269$). There was no difference between the frequency of pathogens detected on tables, plates, floors, or hands.

Association between domestic hygiene practices and enteric pathogens

Treatment of drinking water was associated with a reduction in the frequency of a pathogen detected in drinking water (p=0.0098), with no pathogens being found in any drinking water samples that were treated (Table 2.1). 70% of households reported ever treating their drinking water, and 42.5% of households had treated the drinking water currently stored in their household that was sampled (25% with liquid chlorine, 12.5% by boiling, and 5% with tablet chlorine). Chlorine treatment often did not meet recommended guidelines, as there was no free chlorine residual detected in sample water from 60% of households that had treated their water with liquid chlorine, likely due to water storage exceeding the recommended 24 hours (reported storage times for households with no residual ranged from 22.5 – 90 hours). However despite non-optimal treatment, pathogens were still not detected in any treated drinking water sampled, suggesting no recontamination of water by hands or utensils in these households. Liquid chlorine had been distributed to many households in the study area due to the cholera outbreak, which is likely the reason for the relatively high percentage of households treating their water it.

The time since last hand washing was found to be associated with pathogen detection for caregiver's hands and children's hands, with more associations observed for children's hands (Table 2.1). For caregivers, higher *Shigella* spp./EIEC counts were associated with more than one hour having past since last washed hands (p=0.0376), although no associations were observed for other pathogens. For children, the detection of *C. jejuni*, *V. cholerae,* and multiple pathogens were each associated with time since hands were last washed, with larger amounts of time associated with greater detection of the pathogens (Table 2.1). The average amount of time since last hand washing was also much longer for children than it was with caregivers, which

might have explained why there were more correlations between pathogen detection and time since last wash for children. On average, 3.3 hr (SD 4.9, range 0.3-23.5) for caregivers and 9.7 hr (SD 5.6, range 0.5-23) for children had past since last hand wash at the time of sample collection. Although times since last hand wash are rarely reported for young children, the time since last handwash reported for caregivers is similar to other studies in nearby Tanzania: one which found an average of 3 hr had past since the last handwash with soap,[29] and one which found a median time of 1-4 hr since their last handwash among adults.[30] Our results suggest that a greater frequency of hand washing for children's hands could reduce pathogen contamination of their hands.

Other domestic hygiene practices had no associations with pathogen detection. The time since the table was last cleaned or whether there was visible dirt on the table were not associated with the detection of at least one pathogen or the presence of multiple pathogens on the table (Table 2.1). Similarly, the time since the floor was last cleaned was also not associated with the detection of at least one pathogen or the presence of multiple pathogens on the floor (Table 2.1). This suggests that the existing cleaning methods may be inadequate for removing pathogens from tables and floors, although the lack of association may also have been due to the small sample size of tables and floors sampled (n=28 for tables and n=24 for floors). The majority of households reported using either water, soap, and a cloth or a wet cloth to clean tables and other household surfaces and either water, soap, and a mop or water and a mop to clean floors, and no households reported using bleach or a similar disinfectant for cleaning household surfaces or floors. The promotion of bleach or a similar disinfectant may be an effective method for reducing pathogen contamination on household surfaces and floors.

Similarly, no association was found between at least one pathogen being detected or multiple pathogens detected with adult household members using a toilet connected to the sewer line vs. a latrine (Table 2.1). This result may be due to the densely populated nature of the study site, because it was not uncommon for a latrine to be in or near compounds that also had access to a toilet connected to a sewer line.

Associations between chicken ownership and *Campylobacter jejuni*

C. jejuni was detected in households with chickens more often than in household without chickens ($p<0.0001$; Table 2.1). *C. jejuni* was also detected in floor and soil samples for households than owned chickens more often (floor: $p<0.0001$, soil: $p=0.0014$). Additionally, the log count of *C. jejuni* gene copies detected in soil samples was higher in households with chickens ($p=0.0447$). These results suggest that *C. jejuni* is likely entering the home environment due to chicken feces associated with livestock ownership, both on the soil outside of households, and on floors inside homes, likely due to the bacteria being carried into the home by foot traffic or from chicken entering the home. These results suggest that poor management of chicken feces could lead to *C. jejuni* exposure, negatively impacting child health. This is supported by a recent systematic review, which found a significant association between domestic exposure to poultry and human diarrhea caused by *Campylobacter* spp.[31] A study in Ecuador also found that *C. jejuni* was the most commonly detected *Campylobacter* species in children and chicken feces and identified the same *C. jejuni* strains in isolates from the feces of both.[19] Better containment and management of chicken feces could help reduce *C. jejuni* contamination and associated illness in this community.

Relative important of pathogens at different exposure points

The high frequency of detection of *C. jejuni* and *Shigella* spp./EIEC along transmission pathways is notable as these pathogens have been implicated as causes of bloody diarrhea,[11] and have been associated with gut inflammation characteristic of EED[12] and linear growth faltering.[32–34] Both of these pathogens were detected at high frequencies in soil samples, indicating that soil ingestion may be an important route of transmission for these pathogens. High rates of soil ingestion were previously found in this study population, including a strong association between soil ingestion and diarrhea.[23] The high levels of these pathogens in soil demonstrates the importance of education and interventions to reduce soil ingestion by young children, as the presence of these pathogens demonstrate that ingesting soil in this study area could potentially lead to diarrhea in the short-term and EED and growth faltering in the long-term. However, *C. jejuni* and *Shigella* spp./EIEC were also detected at a number of different exposure points including on hands, on floors and tables in households, and in drains and standing water in compounds. The frequent detection of *C. jejuni* and *Shigella* spp./EIEC suggests that children are constantly being exposed to these two pathogens, and more comprehensive interventions that prevent transmission from a number of different exposure points might be required to prevent continued exposure to these pathogens.

The moderately high frequency of detection of *V. cholerae* in almost all sample types is likely explained by the study occurring during a cholera outbreak. While the infectious dose in healthy volunteers is often considered high (10^8-10^{11} cells), this dose can drop to 10^4-10^8 cells when stomach acid is neutralized by a meal.[35] However, even more concerning in the context of a

cholera outbreak in a densely populated setting is that the infectious dose might drop even lower when *V. cholerae* is hyperinfective, which occurs for at least five hours after the cells are passed into the environment.[35] Although blocking the water transmission pathway is typically targeted during cholera outbreaks due to the high levels of *V. cholerae* that can be present as a result of the bacteria aggregating on copepods, our study suggests that interventions targeting other pathways could potentially help reduce transmission rates in densely populated settings. In addition to water, we also detected *V. cholerae* on hands and fomites in the household, so promotion of handwashing and disinfecting household surfaces could also be beneficial, as well as additional protections to limit interactions with soil and drains.

Our study also provides more evidence of the importance of hand to mouth contacts for enteric disease transmission. Although it is not uncommon for studies to measure fecal indicator bacteria on child hands, measurement of enteric pathogens on children's hands are rare. Our finding that at least one enteric pathogen was detected on 37% of children's hands, and that all pathogens we measured were detected on child hands, provides additional evidence for the importance of this transmission pathway. Young children in developing countries have frequent hand to mouth contacts, with a recent study in rural Bangladesh observing a median hand to mouth contact frequency of 30 contacts/hour for the oldest group observed (12-18 months old) up to 37 contacts/hour for the youngest observed group (3-6 months old).[36] The high number of hand to mouth contacts, combined with a transfer efficiency from hands to the mouth area ranging from approximately 33-41%,[37] lead to an important transmission pathway of enteric disease.

Our results are generally consistent with the few studies that have measured pathogen contamination in low-income countries. A study in Tanzania that evaluated water and hands as exposure points found that viral pathogen genes were most frequently detected on hands, whereas bacterial pathogen genes were likely to be detected in both water and hands.[29] Similarly, we only detected bacterial pathogens in household water, whereas we detected bacterial and viral pathogens on hands at similar frequencies. Another study in Tanzania found bacterial pathogen genes in soil and on household surfaces, but a low frequency of detection of enterovirus and rotavirus genes in soil samples and no detection of these viral genes on surface samples. Our study detected both bacterial and viral genes in soil and on surfaces at moderately high frequencies. In addition to a difference between study locations, the difference could also be attributed to our study using adenovirus, which is a more persistent DNA virus than the RNA viruses used in the Tanzania study. In a similar urban slum in Uganda, another study found high frequency of adenovirus detection in drains and surface water,[38] similar to what we see in our study. Our study adds to this existing literature by measuring the pathogen contamination for a number of key pathogens at multiple points of exposure both inside and outside the household in an urban setting, identifying differential levels of contamination for these exposure points.

Interactions among transmission pathways

We found strong correlations between pathogen contamination on child and caregiver hands for pairs in the same household (Table 2.2). The detection of any pathogen on a caregiver's hands was associated with the detection of a pathogen on child's hands ($p=0.014$), as was the detection of multiple pathogens on caregiver hands associated with the detection of multiple pathogens on child's hands ($p=0.027$). There were also strong linear correlations between log copies of target

genes on caregiver and child hands for Adenovirus, *C. jejuni*, and *V. cholerae* (Table 2.2). This could be due to children and adults in the same household being exposed to similar fomites and soil, as well as transfer of pathogens from child and caregiver hands to the other when they come in contact with each other. Our results suggest that sampling caregiver hands should give a good representation of the pathogens on child hands, and it may not be necessary to sample both in future studies. With the exception of *Shigella* spp./EIEC, there was also a stronger correlation between pathogen concentrations on child and caregiver hands than for *E. coli* (Table 2.2).

We also found evidence of interactions among other transmission pathways including soil-hands, soil-floor, soil-open drainage ditch/standing, floor-hands, open drainage ditch/standing water-hands, hands-table, hands-water, (Figure 2.2). Detection of a pathogen on water was correlated with detection of a pathogen on hands in the same household. Although we cannot confirm the direction of the association, due to evidence from other studies regarding contamination during storage, it is likely that the association that we saw between these two pathways was due to hands contaminating water. We also saw a relationship between the hands and fomites pathways. Detection of a pathogen on the table surface was correlated with detection of a pathogen on hands in the same household ($p=0.0381$), and associations between the detection of adenovirus and Shigella spp./EIEC on a table and on hands in the same household (Figure 2.2, Table A.5). Additionally, the log gene count on caregiver hands was correlated with the log gene count on tables for Adenovirus, *C. jejuni*, and *Shigella* spp./EIEC ($r_p=0.451$, 0.453, and 0.426 and $p=0.024$, 0.023 and 0.034, respectively). Again we could not assess the direction of this transmission, but it is possible that this correlation is both due to hands contaminating the table and the table contaminating the caregiver's hands. There was also a likely interaction between

the soil and floor transmission pathways, with all floor samples from finished flooring. The detection of Adenovirus, *C. jejuni*, and *Shigella* spp./EIEC on soil outside the household were all associated with the detection of each pathogen on the floor inside the household (Figure 2.2). The log gene count on soil was also correlated with the log gene count on floors for Adenovirus and *C. jejuni* (r_p=0.559, and 0.779 and p=0.008 and <0.0001, respectively). Again, while we cannot confirm the direction of this association, as soil contained higher concentrations of pathogens than were on the household floor, it is likely that this correlation is due to soil being transported into households including pathogens found in the soil. This suggests that either removing shoes or cleaning shoes (or feet if no shoes are worn) before entering the household could be a low-cost method to reduce number of pathogens introduced inside these households. There were also a likely interaction between soil-hands, drainage ditch/standing water-hands, and floor-hands, and we expect that hands are becoming contaminated by contact which each of these due to the higher count of pathogens found in soil, drainage ditch/standing water, and floor samples compared to hands.

Correlations between *E. coli* and enteric pathogen contamination

All pathogen counts showed some level of significant correlation with *E. coli* counts, however, the level of correlation differed among pathogens (Table 2.3). When grouping all samples together for analysis, *Shigella* spp./EIEC showed the strongest correlation (Pearson's r=0.652, p<0.0001), which is unsurprising as *Shigella* spp. is closely related to *E. coli*. *C. jejuni*, another bacterial pathogen, also showed a high level of correlation with *E. coli* (Pearson's r=0.6109, p<0.0001). However, Adenovirus, a viral pathogen, and *V. cholerae*, a bacterial pathogen often

transmitted in clusters on copepods, were less strongly correlated (Pearson's r=0.385 and 0.367 respectively, both $p<0.0001$).

When separating samples out by sample material type, there were differential correlations between pathogens and *E. coli* for different sampling material (Table A.6, SI), suggesting that when *E. coli* is used to measure fecal contamination on a variety of exposure points with different sampling media in the same study, the differential correlation between *E. coli* and pathogens on different sample types could result in a misrepresentation of the relative importance of different exposure points/transmission pathways. For our data, log *E. coli* CFU correlated with detection of a pathogen in water ($p=0.012$) and drains ($p=0.038$), but not on hands or surfaces ($p>0.05$). However, log *E. coli* CFU correlated well with the detection of multiple pathogens on hands ($p=0.012$), surfaces ($p=0.002$), and in drains ($p=0.037$), but not in soil ($p>0.05$) (full results in Table A.6 in SI). When testing for correlation between log *E. coli* and log gene copy counts for individual pathogens by sample type, no correlations were found for water samples or drain samples. For hand samples, *C. jejuni* and *Shigella* spp./EIEC had moderate correlations with *E. coli* counts (Pearson's r=0.316 and 0.321, and p=0.01 and 0.009, respectively). For surface samples, Adenovirus and *C. jejuni* had moderate correlations with E. coli counts (Pearson's r=0.304 and 0.287, and p=0.016 and 0.022, respectively). Finally, only *V. cholerae* has a correlation with *E. coli* in soil (Pearson's r=0.506, p=0.023).

Although we have assumed all pathogens in this analysis have one copy of the target gene per organism, *Shigella* spp. could have multiple copies of *ipaH* in a genome. It should also be noted that only samples that were positive for *E. coli* were analyzed for pathogens, so these

correlations do not refer samples that could be positive for a virus, but not *E. coli*. Another limitation of this analysis is that the recovery efficiency of pathogens from different samples types using qPCR could also vary due to variations in quantity of sample assayed, extraction efficiency, and inhibition.

Study significance and limitations

The pathogen measurements untaken as a part of this study could provide better pathogen exposure data for future quantitative microbial risk assessment (QMRA) analyses, particularly in urban slum settings. Currently, there is very limited data measuring pathogens for many of these exposure points, and few to no studies that looked at these specific and important enteric pathogens at these common exposure points in an urban slum setting. Due to the lack of data, most QMRAs in low-income countries are currently either based on fecal indicators, which may not be well correlated with actual pathogen exposure, or the analysis excludes potentially important exposure points such as hands and fomites.

This study has some limitations. First, due to the cross-sectional nature of the study which collected all environmental samples and household survey data at the same time, causal relationship between associated factors could not be assessed. The samples were also collected during wet season, and a different distribution of pathogens may be present during dry season. Additionally, this study measured four enteric pathogens that are important in diarrheal and EED diseases, but there are several additional enteric pathogens important in disease transmission that were not included in this study. We also used qPCR to detect and quantify pathogens, so we could not verify whether or not the pathogens were in an infectious state at the time of sampling,

although the detection of pathogen DNA demonstrates that infectious pathogens are likely to be transmitted through that exposure point, even if they were not infectious at the time of sampling. Furthermore, the potentially important exposure route of foods consumed and flies were excluded from this study.

Despite its limitations, this study provides evidence that hands, household fomites and floors, soil, drainage ditches, standing water, and streams are important exposure points for enteric pathogens in urban slums. Water was also considered an exposure point for *V. cholerae* during the cholera outbreak, but a pathogen other than *V. cholerae* was only detected in the stored water of one household, so water may not be as important of an exposure point for other enteric pathogens in this setting. Our results also suggest that the most effective interventions in this community, and potentially other densely populated urban slum communities, are likely interventions that can disrupt many transmission pathways since pathogens were frequently detected at multiple exposure points so single interventions may not adequately reduce pathogen exposure.

2.5 Acknowledgements

The authors thank Phantus Wambiya, Rodah Obondi, and Philip Omandid for their help conducting household interviews and fieldwork, Gabrielle Levato for her help with field sampling and lab work, and Sanergy for providing fecal samples collected from their urine-diversion toilets. We also thank the participating households who made this study possible. Valerie Bauza was supported by the National Science Foundation Graduate Research Fellowship Program under grant no. DGE-1144245 while conducting this work, and travel and supplies were

supported by the Safe Global Water Institute (SGWI) and the Department of Civil and Environmental Engineering at the University of Illinois at Urbana-Champaign.

Table 2.1. Associations between pathogen detection and survey data.

Descriptive variable	Outcome variable	Mean (SD)	p-value	Conclusion
Water				
Water treatment	Pathogen detected	Treated: 0 (0) Not treated: 0.31(0.48)*	0.0098	Pathogens less likely to be detected in treated water
Hands				
Caregiver hands washed in past 1 hour	*Shigella* spp./EIEC count (log gene copies)	Washed: 2.35 gc (0.30) Not washed: 2.65 gc (0.66)	0.038	Hands washed recently were more likely to have lower *Shigella*/EIEC count
C. jejuni detected on child's hands	Time since last wash	Detected: 12.5 hr (0.5) Not detected: 9.4 hr (5.8)	0.011	A longer time since last hand wash was associated with the detection of *C. jejuni*, *V. cholerae*, and multiple pathogens on children's hands
V. cholerae detected on child's hands	Time since last wash	Detected: 12.4 hr (0.5) Not detected: 9.3 hr (5.9)	0.014	
Multiple pathogens detected on child's hands	Time since last wash	Detected: 12.2 hr (0.3) Not detected: 9.5 hr (5.8)	0.020	
Table Surface				
Pathogen detected on table	Time since last clean	Detected: 11.5 hr (7.3) Not detected: 8.5 hr (4.5)	0.24	No association between pathogens on table and time since last cleaning
Multiple pathogens detected on table	Time since last clean	Detected: 10.8 hr (6.4) Not detected: 9.9 hr (6.2)	0.82	
Dirt visible on table	Pathogen detected	Detected: 0.55 (0.5) Not detected: 0.50 (0.5)*	0.42	No association between pathogens on table and visible dirt
Dirt visible on table	Multiple pathogens detected	Detected: 0.27 (0.3) Not detected: 0.07 (0.5)*	0.11	
Floor				
Pathogen detected on floor	Time since last clean	Detected: 13.1 hr (8.2) Not detected: 12.9 hr (7.1)	0.52	No association between pathogens on floor and time since last cleaning
Multiple pathogens detected on floor	Time since last clean	Detected: 13.6 hr (7.4) Not detected: 12.0 hr (9.0)	0.35	
Households with chickens and Campylobacter jejuni detection				
Household with chickens	*C. jejuni* inside household	Chickens: 1.0 (0) No chickens: 0.34 (0.5)*	<0.0001	Chickens in household were associated with *C. jejuni* detected in household, on floor, and in soil and a higher count of *C. jejuni* count in soil
Household with chickens	*C. jejuni* on floor	Chickens 1.0 (0) No chickens: 0.33 (0.5)*	<0.0001	
Household with chickens	*C. jejuni* in soil	Chickens: 1.0 (0) No chickens: 0.72 (0.5)*	0.0014	
Household with chickens	*C. jejuni* count in soil (log gene copies)	Chickens: 3.21 gc (0.8) No chickens: 3.97 gc (0.8)	0.045	
Households using toilet with sewer connection				
Household using toilet with sewer connection	Pathogen in household, on soil, or in drainage ditch	Sewer: 0.91 (0.3) No sewer: 0.94 (0.2)*	0.74	No association between household using a toilet with a piped sewer connection and pathogen detection

* Frequency of detection (0 was assigned to non-detect samples and 1 to samples with positive detection)

Table 2.2. Associations between of *E. coli* and pathogen detection and quantification among pairs of caregiver and child hands in the same households.

Outcome variable	Mean (SD) or test statistic	p-value	Conclusion
Associations between caregiver hands and child hands			
Pathogen detection	Fisher's exact test (FET)	0.014	Association between finding a pathogen on a child's hand and a caregiver's hand
Multiple pathogens detected	FET	0.027	Association between finding multiple pathogens on a child's hand and a caregiver's hand
Adenovirus detection	FET	0.01	Association between finding Adenovirus on a child's hand and a caregiver's hand
Log CFU *E. coil*	Pearson's r = 0.505	0.007	*E. coli*, adenovirus, *C. jejuni*, and *V. cholera* counts correlated between child and caregiver hands
Log gene copies for Adenovirus	Pearson's r = 0.795	<0.0001	
Log gene copies for *C. jejuni*	Pearson's r = 0.80	<0.0001	
Log gene copies for *V. cholerae*	Pearson's r = 0.635	0.0005	
Log CFU *E. coli*	Caregiver: 2.6 CFU (0.6) Child: 2.3 CFU (0.7)	0.0114	*E. coli* counts on caregiver hands greater than counts on child hands

Table 2.3. Associations between log *E. coli* CFU and pathogen data.

Pathogen measurement	Mean (SD) or Pearson's r	p-value
Pathogen detected	No: 1.29 (0.93) Yes: 2.52 (1.90)	<0.0001
Multiple pathogens detected	No: 1.38 (1.02) Yes: 3.87 (1.87)	<0.0001
Log (gene copies Adenovirus)	Pearson's r = 0.3854	<0.0001
Log (gene copies *C. jejuni*)	Pearson's r = 0.6109	<0.0001
Log (gene copies *Shigella*/EIEC)	Pearson's r = 0.6516	<0.0001
Log (gene copies *V. cholera*)	Pearson's r = 0.3672	<0.0001

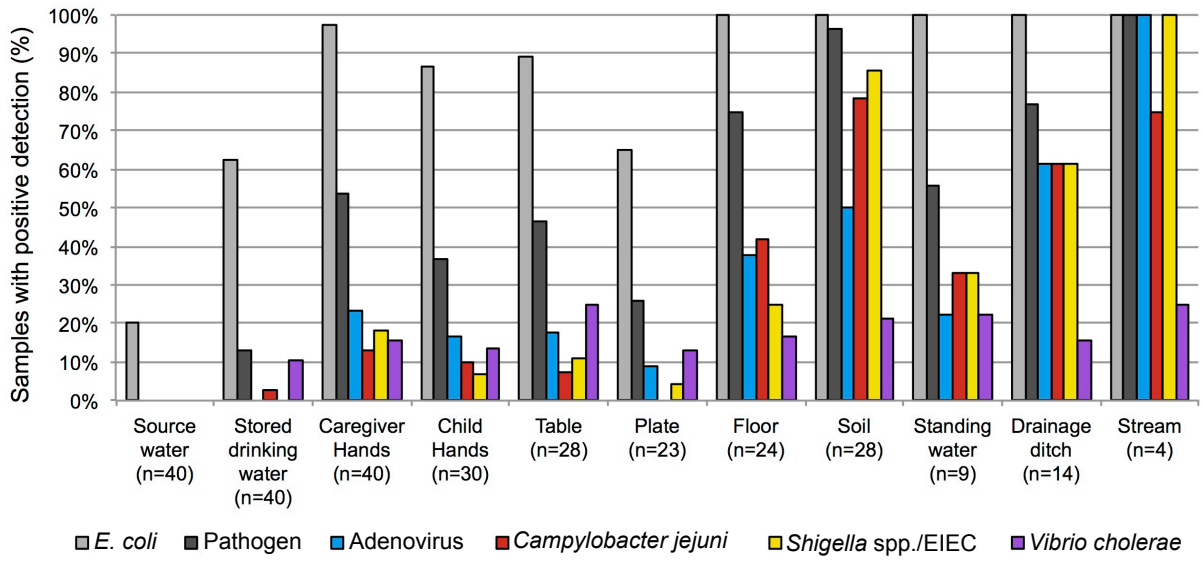


Figure 2.1. Frequency of detection of *E. coli* and enteric pathogens at different exposure points. This graph shows the percent of samples with detectable levels of *E. coli* (gray), any of the four enteric pathogens (black), adenovirus (blue), *Campylobacter jejuni* (red), *Shigella* spp./EIEC (yellow), and *Vibrio cholera* (purple). The mean and standard deviation for the quantified counts of E. coli colony forming units and pathogen gene copies at each sample location are reported in Table A.4 (SI).

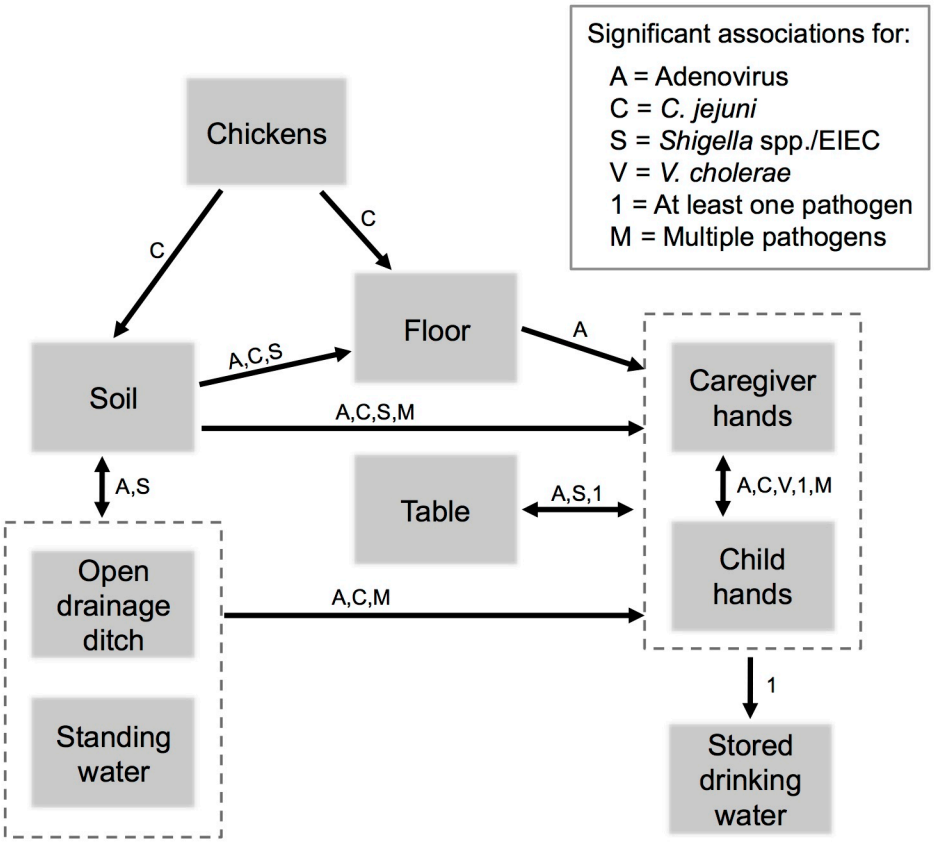


Figure 2.2. Associations between pathogen detection at exposure points that suggests interactions among transmission pathways. Significant associations ($p<0.05$) between two exposure points are shown for adenovirus, *Campylobacter jejuni*, *Shigella* spp./EIEC, *Vibrio cholera*, any of the four enteric pathogens, and multiple enteric pathogens detection. Arrows indicate the likely direction of interactions. Table A.5 provides more details for statistical test results for each association.

2.6 References

1. World Health Organization. Diarrhoeal disease fact sheet. *WHO* (2017). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/. (Accessed: 6th June 2017)
2. Humphrey, J. H. Child undernutrition, tropical enteropathy, toilets, and handwashing. *The Lancet* 374, 1032–1035 (2009).
3. Korpe, P. S. & Petri, W. A. Environmental enteropathy: critical implications of a poorly understood condition. *Trends Mol. Med.* 18, 328–336 (2012).
4. Lunn, P. G. The impact of infection and nutrition on gut function and growth in childhood. *Proc. Nutr. Soc.* 59, 147–154 (2000).
5. Prendergast, A. & Kelly, P. Enteropathies in the Developing World: Neglected Effects on Global Health. *Am. J. Trop. Med. Hyg.* 86, 756–763 (2012).
6. Mbuya, M. N. & Humphrey, J. H. Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries. *Matern. Child. Nutr.* 12, 106–120 (2016).
7. Checkley, W. *et al.* Multi-country analysis of the effects of diarrhoea on childhood stunting. *Int. J. Epidemiol.* 37, 816–830 (2008).
8. Black, R. E. *et al.* Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet* 382, 427–451 (2013).
9. Guerrant, R. L., DeBoer, M. D., Moore, S. R., Scharf, R. J. & Lima, A. A. M. The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease. *Nat. Rev. Gastroenterol. Hepatol.* 10, 220–229 (2012).
10. Kotloff, K. L. *et al.* Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet* 382, 209–222 (2013).
11. Platts-Mills, J. A. *et al.* Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob. Health* (2015).
12. Kosek, M. N., Investigators, M.-E. N. & others. Causal Pathways from Enteropathogens to Environmental Enteropathy: Findings from the MAL-ED Birth Cohort Study. *EBioMedicine* 18, 109–117 (2017).
13. Curtis, V., Cairncross, S. & Yonli, R. Review: Domestic hygiene and diarrhoea–pinpointing the problem. *Trop Med Int Health* 5, 26–30 (2000).
14. Exley, J. L. R., Liseka, B., Cumming, O. & Ensink, J. H. J. The Sanitation Ladder, What Constitutes an Improved Form of Sanitation? *Environ. Sci. Technol.* 49, 1086–1094 (2015).
15. Clasen, T. F. *et al.* Interventions to improve disposal of human excreta for preventing diarrhoea. in *Cochrane Database of Systematic Reviews* (ed. The Cochrane Collaboration) (John Wiley & Sons, Ltd, 2010).
16. Brown, J., Cairncross, S. & Ensink, J. H. J. Water, sanitation, hygiene and enteric infections in children. *Arch. Dis. Child.* 98, 629–634 (2013).
17. Majorin, F. *et al.* Child Feces Disposal Practices in Rural Orissa: A Cross Sectional Study. *PLoS ONE* 9, e89551 (2014).
18. Bauza, V. & Guest, J. S. The effect of young children's faeces disposal practices on child growth: Evidence from 34 countries. *Trop. Med. Int. Health* (2017).
19. Vasco, K., Graham, J. P. & Trueba, G. Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in ecuador. *Appl. Environ. Microbiol.* 82, 4218–4224 (2016).

20. Ishii, S. & Sadowsky, M. J. Escherichia coli in the environment: implications for water quality and human health. *Microbes Environ.* 23, 101–108 (2008).
21. Savichtcheva, O. & Okabe, S. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Res.* 40, 2463–2476 (2006).
22. Harwood, V. J. *et al.* Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Appl. Environ. Microbiol.* 71, 3163–3170 (2005).
23. Bauza, V., Ocharo, R. M., Nguyen, T. H. & Guest, J. S. Soil Ingestion Is Associated with Child Diarrhea in an Urban Slum of Nairobi, Kenya. *Am. J. Trop. Med. Hyg.* 96, 569–575 (2017).
24. Jothikumar, N. *et al.* Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Appl. Environ. Microbiol.* 71, 3131–3136 (2005).
25. Ishii, S., Segawa, T. & Okabe, S. Simultaneous Quantification of Multiple Food and Waterborne Pathogens by Use of Microfluidic Quantitative PCR. *Appl. Environ. Microbiol.* AEM.00205-13 (2013). doi:10.1128/AEM.00205-13
26. Blackstone, G. M. *et al.* Use of a real time PCR assay for detection of the ctxA gene of Vibrio cholerae in an environmental survey of Mobile Bay. *J. Microbiol. Methods* 68, 254–259 (2007).
27. Cao, Y., Griffith, J. F., Dorevitch, S. & Weisberg, S. B. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *J. Appl. Microbiol.* 113, 66–75 (2012).
28. Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55, 611–622 (2009).
29. Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J. & Boehm, A. B. Hands and Water as Vectors of Diarrheal Pathogens in Bagamoyo, Tanzania. *Environ. Sci. Technol.* 47, 355–363 (2013).
30. Pickering, A. J., Boehm, A. B., Mwanjali, M. & Davis, J. Efficacy of Waterless Hand Hygiene Compared with Handwashing with Soap: A Field Study in Dar es Salaam, Tanzania. *Am. J. Trop. Med. Hyg.* 82, 270–278 (2010).
31. Zambrano, L. D., Levy, K., Menezes, N. P. & Freeman, M. C. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. *Trans. R. Soc. Trop. Med. Hyg.* 108, 313–325 (2014).
32. Platts-Mills, J. A. *et al.* Association between enteropathogens and malnutrition in children aged 6–23 mo in Bangladesh: a case-control study. *Am. J. Clin. Nutr.* 105, 1132–1138 (2017).
33. Black, R. E., Brown, K. H. & Becker, S. Effects of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh. *Pediatrics* 73, 799–805 (1984).
34. Lee, G. *et al.* Effects of Shigella-, Campylobacter-and ETEC-associated diarrhea on childhood growth. *Pediatr. Infect. Dis. J.* 33, 1004–1009 (2014).
35. Nelson, E. J., Harris, J. B., Morris Jr, J. G., Calderwood, S. B. & Camilli, A. Cholera transmission: the host, pathogen and bacteriophage dynamic. *Nat. Rev. Microbiol.* 7, (2009).

36. Kwong, L. H. *et al.* Hand-and object-mouthing of rural Bangladeshi children 3–18 months old. *Int. J. Environ. Res. Public. Health* 13, 563 (2016).
37. Rusin, P., Maxwell, S. & Gerba, C. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J. Appl. Microbiol.* 93, 585–592 (2002).
38. Katukiza, A. Y., Temanu, H., Chung, J. W., Foppen, J. W. A. & Lens, P. N. L. Genomic copy concentrations of selected waterborne viruses in a slum environment in Kampala, Uganda. *J. Water Health* 11, 358–370 (2013).

CHAPTER 3: MICROBIAL SOURCE TRACKING OF YOUNG CHILDREN'S FECES: EVALUATION OF METHODS AND ENVIRONMENTAL FECAL CONTAMINATION IN KENYA[†]

3.1 Abstract

Child exposure to fecal contamination often remains common in low-income countries after improvements to toilet/latrine infrastructure, and unsafe disposal of children's feces likely contributes to this exposure. However, the relative importance of child feces disposal to environmental fecal contamination is not well understood. In this study, we evaluated microbial source tracking methods using bacterial community sequencing for tracking young children's feces (<3 years old) separately from other human-associated fecal contamination sources, and found that the SourceTracker software tool identified young children's feces separately from older child/adult feces with a high level of sensitivity and specificity in spiked water and soil samples using sequencing data from V3-V4 or V4 regions of the 16S rRNA bacterial gene. This method was then used to evaluate human fecal contamination in environmental samples (drinking water, caregiver and child hand rinses, surface swabs, soil, open drainage ditches, standing water, and streams) collected from 40 households in the urban slum of Kibera, in Nairobi, Kenya. Young children's feces was found to contaminate all sample types except for drinking water, although child feces tended to be the dominant source of human fecal contamination inside households (hands and surfaces), older children/adults tended to dominate

[†] This chapter is in preparation: Bauza, V., Madadi, V., Ochao, R. M., Nguyen, T.H., Guest, J.S. Microbial source tracking of young children's feces: Evaluation of methods and environmental fecal contamination in Kenya. To be submitted to *Environmental Science and Technology.*

human fecal contamination outside households (standing water and streams) and both dominated human contamination in open ditches.

3.2 Introduction

Diarrheal disease, commonly caused by exposure to fecal pathogens, is the second leading cause of mortality in children under five, resulting in approximately 525,000 deaths annually.[1] In addition to causing diarrhea, repeated exposure to fecal contamination may also lead to environmental enteric dysfunction and stunting, which may increase the risk of mortality[2,3]. Human fecal contamination often enters the environment due to poor sanitation practices, and is then spread through the fecal-oral pathway with typical exposure points of water, fields/floor, hands, food, and flies.[4] Measures to improve sanitation practices and reduce fecal contamination often focus on open defecation reduction and toilet/latrine installation. However, these interventions tend not to address the sanitation needs of children given that many are too young to use a toilet or latrine themselves, and children are commonly observed to not use latrines after installation[5–7] or to perform anal cleansing outside of the latrine[5]. As a result, unhygienic practices related to young children's feces may contribute to fecal contamination of the domestic environment even if the household has access to a toilet facility. Understanding the relative contribution of young children's feces to domestic fecal contamination as well as how exposure to young children's feces differs among exposure points could help better target sanitation and hygiene interventions to reduce exposure to fecal contamination. However, to our knowledge, there is currently no reliable method to differentiate between fecal contamination from young children's feces and other human sources.

Fecal contamination is typically monitored using fecal indicator bacteria, such as *Escherichia coli*, total coliforms, fecal coliforms, and enterococci.[8] However, fecal indicator bacteria are not specific to one source of pollution and can originate from the feces of humans and animals, as well as from non-fecal sources[9]. Leveraging specific genetic markers and library-independent methods, microbial source tracking is commonly used to identify fecal contamination, distinguishing among human and animal (e.g., ruminant, cow, gull, dog) feces.[9] Alternatively, microbial community source tracking methods leverage the diversity of microbial communities to establish a signature for each source,[10] enabling the identification of contamination sources that can not be identified by a specific marker gene,[11] and have been used to identify fecal contamination in waterways.[10–14] Since microbial communities vary among different sources of human waste (e.g., human feces, septic tanks, sewage), community-based methods can also be used for differentiating between human sources.[15] Although these methods have not been previously used to discern fecal contamination from young children, Yatsunenko, et al.[16] demonstrated that the gut microbiome undergoes large changes in bacterial diversity in the first three years of life, and that there are large differences (measured by UniFrac distance) between the fecal microbial community of children under three years old and adults. Conveniently, three years old is also the age at which parents may start to think it is appropriate for children to use a latrine or flush toilet.[17] As children under three years old would be unlikely to use a toilet facility themselves, which has also been observed in a number of low- and middle-income countries,[18,19] their feces must be managed separately by caregivers or it will be left in the place of defecation. Therefore, we hypothesize young children's feces (from children under three years old) can be identified and tracked separately from older child/adult feces using microbial community source tracking methods. To our knowledge, no previous studies have proposed a microbial source

tracking method to identify fecal contamination resulting from unhygienic child feces management or assessed the relative contribution of young children feces to domestic fecal contamination from humans.

The objectives of this work are to: (1) evaluate the use of microbial community methods for source tracking young child (< 3 years old) fecal sources separately other human-associated fecal sources, and (2) leverage this method to characterize the contribution of fecal contamination from young children at different potential exposure points (including hands, household surfaces, soil, drinking water, open drainage ditches, and standing water) in an urban slum environment. We validated these microbial source tracking methods for water and soil samples spiked with fecal sources using information generated from high-throughput sequencing along with the use of computational microbial source tracking (MST) tools, such as SourceTracker[20]. We evaluated the performance of the methods for identifying the dominant source of contamination and the presence/absence of each contamination source using two different primer sets targeting different regions of the 16S rRNA gene in bacteria. We then used the validated source tracking methods to analyze the source of human fecal contamination in environmental samples collected from the domestic environment and nearby outdoor locations from households in Kibera urban slum in Kenya. Ultimately, this work will enable the characterization of the contribution of young child feces (relative to other human fecal sources) in domestic environments to better target limited development resources and achieve greater reductions in fecal contamination exposure.

3.3 Materials and Methods

Note: Many of the methods related to household selection, sample collection, *E. coli* enumeration, and genomic DNA extraction have already been given in Chapter 2, but they have also been copied here to make it easier to understand the methods used in this chapter.

Study site and household selection

This study was conducted in the Kibera urban slum in Nairobi, Kenya in June 2015. Households in the study site were clustered into compounds that shared outdoor open space and usually shared the same toilet/latrine and water source. Compounds were purposively selected for inclusion in this study from three separate wards (Makina, Sarangombe, and Lindi) in Kibera, in an attempt to increase the diversity of sanitation and drainage infrastructure serving included compounds. Households with children under five in each selected compound were randomly selected for inclusion, with the requirement that at least one household included had to have a child under three for each compound. Once informed consent was completed, household interviews were conducted with the primary caregiver to obtain information about household demographics and water, sanitation, and hygiene behaviors. 55 children under five years old (including 43 children under three years old) were included in this study, from a total of 40 households and 16 compounds. 38 of the 40 households included in the study had a child under three years old. This study was conducted using the same households as Bauza et al.[21] which evaluated the association between child soil ingestion and diarrhea, as well as soil contamination with *E. coli* and a human-associated fecal marker. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign and the National Commission for Science, Technology and Innovation in Kenya.

Fecal sample collection

Sterile polystyrene sampling spoons (Nasco, Fort Atkinson, WI) were used to collect fecal samples from adults, latrines, and children under three. Collected samples were placed in sterile Whirl-Pak bags (Nasco) and transported to the laboratory in a cooler. When a child fecal sample was not available at the time of the initial household visit, a sterile bag and spoon were left at the household with instructions for collecting a fecal sample, which was then picked up by investigators within 24 hours. Fecal samples from older children and adults were collected in aggregate from the feces compartment of urine diversion toilets operated in Nairobi slums by sanitation start-up Sanergy, using visual assessment to select samples of feces from older individuals. In the laboratory, individual fecal samples were homogenized by hand prior to molecular and microbial processing.

Environmental sample collection

Water sampling. Source water and stored household water samples were collected from each household using sterile Whirl-Pak bags. For stored household water samples, the respondent was asked to retrieve water the way she would for a child and pour the water into the bag. The enumerator asked if anything had been done to treat the water, and if so, how long ago the water had been treated. Water was tested for free and total chlorine content prior to sample collection using a chlorine test strip (Hach, Loveland, CO), and water samples that tested positive for chlorine were collected in sterile Whirl-Pak bags containing sodium thiosulfate (to neutralize the chlorine).

Hand rinse sampling. Hand rinse samples were collected from one caregiver and one child at each household. Respondents were asked to place their hands, one at a time, into a 24-oz sterile Whirl-Pak bag containing 150 mL of sterile phosphate-buffered saline (PBS). Each hand was massaged through the bag for 30 seconds. The respondent was asked how much time had past since she washed her hands and since her child's hands had been washed.

Soil sampling. Where applicable, soil samples were collected from the household entrance and/or shared common space near each household. Details of soil sample collection and processing are described in Bauza et al.[21]

Surface sampling. Surface samples were collected from two of the following three items in each household: a table, a plate or bowl, and the floor. Nylon-flocked swabs in liquid Amies elution solution (Eswab, BD, Franklin Lakes, NJ) were used to sample surfaces. Duplicate swab samples were collected at each location. For sampling, the wetted tip of the swab was used to wipe a 10 cm by 10 cm surface, wiping in the horizontal direction with one side of the swab and the vertical direction with the other side of the swab. The swab was placed back in a sterile tube containing 1 mL of Amies elution solution until analysis. At the time of sampling, the surface material, time since last cleaning of surface, temperature, and relative humidity were recorded.

Open drainage ditch and standing water sampling. Any open drainage ditches or large standing water puddles within the compound (or immediately adjacent) were sampled. Sterile polystyrene sampling spoons were used for sample collection and transfer to 15 mL sterile centrifuge tubes (Corning Inc., Corning, NY).

Spiking study

Ambient source water and soil samples from the urban slum were spiked with known fecal sources to evaluate if microbial community analysis could accurately identify fecal contamination sources in these environmental media. Fecal sources included young child feces (from children under three years old), older child/adult feces, pit latrine fecal sludge, and open drainage ditches. Spiked samples were created using methods similar to Cao et al.[15] For feces samples, 0.2 g of homogenized feces was added to between 20 mL and 50 mL of source water, mixed by hand for 2 min to create a slurry, and diluted to the final concentration using additional source water. For the preparation of spiked soil samples, 250 μL of well mixed fecal slurry or raw open drainage ditch water was added to 5 g of soil. For the spiked water samples, fecal slurry was then filtered through 47 mm, 0.45 μm pore size mixed cellulose esters HA filters (Millipore, Billerica, MA), and open drainage ditch samples were mixed by hand, diluted 1:100 (v_{sample}:v_{final}) with source water, mixed by hand again, and filtered. Paired samples containing two spiked fecal sources were also created by mixing 90% of one potential fecal source (by volume) with 10% of another potential fecal source, which resulted in the dominant spiked source contributing 57% to 99% of *E. coli* colony forming units (CFU). The final concentration of fecal sources spiked in water and soil samples ranged from 10^1 to 10^6 CFU *E. coli* per sample. Source water for spiked samples included a variety of different local water sources of varying microbial quality to evaluate if this method could be used to determine the fecal source of post-supply water contamination, and the soil used for spiked samples was collected from a location in Kibera that was near visited households but unlikely to be contaminated. In total, 30 spiked water samples and 10 spiked soil samples were created and analyzed.

E. coli enumeration

E. coli was enumerated using m-ColiBlue24 Broth media (Hach, Loveland, CO) following the manufacturer's protocol approved by the United States Environmental Protection Agency. Plates were incubated at 35^{o}C for 24 hours. *E. coli* were enumerated from a subset of fecal samples after dilution with PBS. 100 mL of each water sample and 10 mL of each hand rinse sample was filtered individually through 47 mm, 0.45 μm pore size mixed cellulose esters filters (Pall Corporation, Port Washington, NY). For soil samples, bacteria were eluted in PBS from 2 g of soil, diluted, and filtered. For surface swab samples, swabs were vortexed in the tube for 20 sec and removed from the tube while pressing the swab against the sides of the tube to recover the liquid in the swab, and the 1 mL of wetting solution was filtered and the tube was rinsed with PBS that was also filtered. For open drainage ditch and standing water samples, a 10^{-3} ($v_{sample} \cdot v_{final}^{-1}$) dilution was filtered. All samples were transported and stored in a cooler with ice until they were processed within 8 hrs of collection.

DNA extraction

Total DNA was extracted from a 0.25 g sample for fecal and soil samples, 1 mL of wetting solution for swab samples, and 1 mL of open drainage ditch and standing water samples within 8 hrs of collection using PowerSoil DNA Isolation kit (MO BIO Laboratories, Inc., Carisbad, CA) following manufacturer's guidelines. Composite fecal samples for young child feces (composite of 10 samples), pit latrine feces (composite of 8 samples), and older child/adult feces (composite of 6 samples) were extracted in duplicate.

For water and hand rinse samples, 250 mL of each water sample and 50 mL of each hand rinse sample was filtered separately through 47 mm, 0.45 μm pore size mixed cellulose esters HA filters (Millipore) for downstream molecular processing. Filters were aseptically rolled into 5 mL transport tubes (Eppendorf, Hamburg, Germany) and frozen at -20^{o}C for up to 3 weeks, transported back to the University of Illinois at Urbana-Champaign in a cooler with ice packs, and stored at -80^{o}C until DNA extraction. DNA was extracted from water and hand rinse filters using PowerWater DNA Isolation kit (MO BIO Laboratories, Inc., Carisbad, CA) following manufacturer's guidelines. DNA was extracted from filters of water samples spiked with fecal sources using PowerSoil DNA Isolation kit.

Molecular analysis for human-specific *Bacteroides* detection

The detection of a human-associated *Bacteroides* fecal marker was used to select environmental samples for microbial community sequencing, because this fecal marker indicates that the presence of human fecal contamination is likely. Briefly, qPCR was used to analyze DNA extracts for the human-associated *Bacteroides* fecal marker HF183 and has been previously reported in Bauza et al.[21] for soil samples. This marker was selected because it has previously been validated in Kenya.[22] 15 μL reactions were used for the HF183 assay, and each reaction contained 1X final concentration of SYBR Green I dye master mix (Applied Biosystems, Waltham, MA) and 250 nM of forward and reverse primers (for detailed methods, see Section B.1 of the Supplementary Information, SI)

PCR and Illumina MiSeq sequencing

The V3-V4 (357F/805R primers) and V4 (515F/806R primers) hypervariable regions of the 16S rRNA gene were amplified by a two-step PCR using a Fluidigm access array integrated fluidic circuit (Fluidigm Corporation, South San Franciso, CA; see Section B.1 of the SI for PCR details).[23] Each primer also contained a Fluidigm adapter, an Illumina linker extension, and a 10-base sample-specific barcode. PCR products were quantified, pooled, and paired-end sequenced (2 x 250bp) on an Illumina MiSeq using V2 chemistry at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. The V3-V4 and V4 regions were selected because these regions are commonly used to analyze the gut microbiome and environmental samples (the V4 region primers are used for the Earth Microbiome Project[24]) and because these primers amplify *Bifidobacterium* well, which we expected to be abundant in young children's feces[16] but is not well amplified by V1-V2 or V1-V3 region primers.[25]

Sequencing data processing

Primers were removed from sequences using Trimmomatic.[26] Paired-end reads were then joined using PEAR,[27] with a minimum of a 30 base pair (bp) overlap for V3-V4 region reads and 200 bp overlap for V4 region reads. V3-V4 reads shorter than 300 bp and V4 reads shorter than 250 bp were discarded. The joined reads were demultiplexed and further processed using QIIME software version 1.9.1.[28] Chimeras were removed using USEARCH version 6.1.[29] Open-reference operational taxonomy unit (OTU) picking was performed to cluster sequences into OTUs using Silva version 128 for reference alignment and taxonomic assignment and the PyNast algorithm for alignment. Sequencing data has been deposited under project reference (TBD once uploaded to NCBI).

OTUs present in less than 1% of samples were filtered and the SourceTracker computational tool (which also you to assign samples as sources and sinks and calculates the proportion of contamination in sink samples from each source)[20] was used as a microbial source tracking tool to identify sources of fecal contamination in environmental (sink) samples. Based on the recommendations of Henry et al.,[10] five runs of SourceTracker were performed for each condition, and presence of each source was determined by calculating the relative standard deviation (RSD; ratio of % standard deviation to predicted proportion (%)) for each source with less than 1% assigned to it. A sample was determine to be positive for a fecal source if at least three of the five runs found that source at a abundance of great than 1% or if the source was positive in at least three runs with an average RSD < 100. As most older child/adult sources were also found to have a proportion of young child feces present, we subtracted this ratio from the estimated proportion of child feces in samples that also identified older child/adult feces. Beta-diversity was also analyzed using Bray-Curtis, weight UniFrac, and unweighted UniFrac measurements. Microbial source tracking was also completed with these distance-based measurements by assigning the fecal source that was most closely related to a sink sample as the dominant source of contamination of that sample. The sensitivity (true positives divided by the sum of true positives and false negatives) and specificity (true negatives divided by the sum of true negatives and false positives) of each method was calculated for each potential source using spiked samples to assess the ability to correctly identify the dominant source of contamination as well as the presence/absence of contamination. Finally, the Bray-Curtis dissimarilty indexes were used to calculate the analysis of molecular variance (AMOVA) using Mothur software version 1.38.1.[30]

3.4 Results and Discussion

Household sanitation and child feces management

All households included in this study reported that adults in the household used a sanitation facility, with slightly more than half using pit latrines compared to toilets that flushed to a sewer system. 50% of households (N=20) used either a pour flush pit latrine or a pit latrine with slab, 42.5% of households (N=17) used a pour flush toilet to sewer, and the remaining 7.5% of households (N=3) used a pit latrine without slab. 95% of toilet facilities that households used were shared with other households, which is consistent with broader observations of high levels of sharing of sanitation facilities in urban slums.[31] Most children were reported to defecate into a potty (31 children; 56.4%) or into a diaper/nappy (19 children; 34.6%), with defecation onto newspaper/napkins or the ground outside (4 children; 7.3%) and use of a toilet/latrine (1 child; 1.8%) being far less common (Figure 3.1). The final disposal location for feces from 23 (41.8%) children was considered safe, being disposed of in the toilet/latrine. The remaining child feces was disposed of in a ditch (23.6%), river or stream (16.4%), garbage (14.6%) or left in the open (3.6%). For children who used potties, 15.4% had wash water (from cleaning the potties) safely disposed of in a toilet/latrine, with 61.6% unsafely disposed of in a ditch, 11.5% on the ground outside the house, 7.7% in a river or stream, and 3.9% near the latrine, demonstrating that potty wash water may be a potential source of fecal contamination even if the child defecates in a potty and feces are disposed of in a toilet/latrine. 97% of caregivers reported always washing their hands with soap and water after using the toilet, but only 33% of caregivers reported washing their hands with soap and water after cleaning a child. Although there is evidence that people tend to over report handwashing behavior,[32,33] there is still a large difference in reported handwashing behavior after each of these two potential exposure activities.

Validation of source tracking methods

The performance of different microbial community source tracking methods were evaluated by analyzing ambient source water and soil samples spiked with one or two fecal sources that included young child feces, older child/adult feces, pit latrine feces, and open drainage ditch liquid. When analyzing spiked samples to identify the dominant source of fecal contamination among these four potential sources, the sensitivity and specificity of the SourceTracker tool and distance-based methods were low, particularly for identifying pit latrine as a source which had a maximum sensitivity in soil samples of 33% using the SourceTracker tool (Table B.2, SI) and 0% using distance-based methods with Bray-Curtis indexes (Table B.3, SI). These methods often incorrectly identified samples that were spiked with pit latrine feces as being dominantly contaminated by older child/adult feces, making these methods unsuitable for separately tracking contamination from older child/adult and pit latrine sources. This finding is unsurprising and likely because samples from pit latrine were predominantly comprised of older child/adult feces and also include young children's feces. These methods were also unsuitable for identifying the presence/absence of contamination when including all of these four potential fecal sources and had particularly poor performance for older child/adult feces and young child feces, with a maximum specificity of 52% for older child/adult feces and 58% for young child feces in water samples using the SourceTracker tool (Table B.4, SI).

Further source tracking analysis only included two potential fecal sources with unique microbial community structures to improve method performance: (i) young children's feces, and (ii) older child/adult feces. Sequence data from ambient soil and extraction blanks for all DNA extraction kits were also assigned in SourceTracker as potential sources to control for potential

contamination. The sensitivity and specificity for identifying the dominant source of contamination and the presence/absence of contamination with young child feces and older child/adult sources in the spiked samples was high for data from both V3-V4 and V4 region primer sets when using SourceTracker (Table 3.1). In fact, SourceTracker performed well in identifying young children's feces as the source of contamination with sensitivities and specificities over 80% (Table 3.1), which has been previously recommended as a cutoff for selecting good source tracking methods.[9] When soil was not included as a potential source in SourceTracker, the sensitivity and specificity were low, particularly for spiked soil samples using V4 region primers. More generally, not all methods worked equally well in water and soil, demonstrating the importance of evaluating source tracking methods separately for more complex media like soil or sediment (instead of only in water), as the performance of community source tracking methods in water may exceed the performance in soil. Among distance-based methods, Bray-Curtis performed the best for identifying the dominant source of contamination, but the performance of these methods was lower than using the SourceTracker tool, with 0% sensitivities for identifying young child feces in soil samples (Table B.3, SI). Due to the high sensitivity and specificity of SourceTracker using V3-V4 region sequencing data with two fecal sources (young children's feces, older child/adult feces), and two other potential contamination sources (ambient soil, extraction blanks) we used this method for all further environmental sample analysis.

Young child vs. older child/adult gut microbiome

The taxa and relative abundance of each taxa present in young children's feces differed from that in older child/adult feces (Figure 3.2, numeric values for relative abundances in Table B.1),

enabling the use of microbial communities for source tracking of young children's feces separately from older child/adult feces. There was a higher diversity of taxa in older child/adult feces compared to young child feces, which is consistent with a previous study of the gut microbiome change with age.[16] Older child/adult feces had an average of 913 OTUs and an average Shannon index of 7.4, compared to young child feces which had an average of 440 OTUs and an average Shannon index of 4.7 using the V3-V4 region primer set of the 16S rRNA gene (excluding taxa that were only present in one sample). Similarly, when the V4 region primer set of the 16S rRNA gene was used older child/adult feces had an average of 1217 OTUs and an average Shannon index of 7.5, compared to young child feces which had an average of 627 OTUs and an average Shannon index of 4.8. Furthermore, the bacterial community structures were found to be significantly different between older child/adult and young child fecal samples using AMOVA for both the V3-V4 and the V4 regions ($p<0.001$).

Abundant taxa found within fecal samples are generally consistent with past gut microbiome studies in sub-Saharan Africa. The bacterial genus *Prevotella* had the highest average relative abundance among genera observed in both young child and older/child adult fecal samples. A high relative abundance of *Prevotella* in the gut microbiome has been associated with a high carbohydrate/low fat diet,[34] and *Prevotella* was previously found in high proportional abundance in feces from children under five years old rural Kenya,[35] was more commonly found in adults from rural Malawi than the United States,[16] and was found to be have a high relative abundance in feces from children 1-6 years old with a high fiber diet in rural Burkina Faso but not in similarly aged European children with a western diet.[36] *Bifidobacterium*, a genus which is often abundant in feces of young children and has been associated with breastfeeding,[16,37,38] had a

higher relative abundance in young child than older child/adult fecal samples (8.0% *vs.* 0.8%). The *Escherichia/Shigella* genus, which are facultative anaerobes and may contain pathogens, was more abundant in young child feces than older child/adult feces (7.6% *vs.* 0.4%). This is consistent with a previous study in rural Kenya that showed a high abundance of organisms in this genus in young children that decreased as children got older.[35] The higher diversity of older child/adult feces was also seen with a high proportion of taxa that were present with relative abundances below 2% compared to young children (45.2% *vs.* 17.3%). There was also more interpersonal variability in the relative abundance of taxa among fecal samples from different young children than fecal samples from different older children/adults (Figure B.2), which is consistent with a previous study's findings that found interpersonal variation of gut bacterial communities was greater among children than adults.[16]

Household and environmental fecal contamination

Samples were prescreened for *E. coli* and the human-associated *Bacteroides* HF183 marker, and only environmental samples positive for both were processed for bacterial community sequencing of the 16S rRNA gene to track young child and older child/adult human fecal sources. Of the 258 total environmental samples collected, 196 were positive for *E. coli* (Figure 3.3), and were screened for the human-associated HF183 marker. 119 of these samples were positive for the human-associated marker (Figure 3.3) and 103 of these samples were sequenced. 18 floor swab samples were positive for the human marker but not sequenced, and one open drain and one soil sample that were not positive for HF183 were sequenced.

Among the sequenced environmental samples, human feces from young children was the dominant source of contamination in the indoor environment (caregiver and child hands, tables, plates) whereas human feces from older children/adults was the dominant source in much of the outdoor environment (standing water, streams) (Figure 3.4A). Both sources of fecal contamination were identified as dominant in an equal number of open drainage ditch samples, and neither was found to be present in drinking water samples that tested positive for HF183 (Figure 3.4A). When examining the presence/absence of fecal sources in HF183-positive samples, child fecal contamination was more prevalent than older child/adult fecal contamination. Specifically, we found child feces was present in over 95% of HF183-positive hand samples, but older child/adult feces was present in less than 10% of samples (Figure 3.4B). We also found child feces to be in almost 70% of HF183-positive table samples, but older child/adult feces was positive in less than 20% of table samples. Although older child/adult feces was more likely to be dominant in HF183-positive environmental samples taken outside of households, child feces was still present in many of these samples (Figure 3.4).

The finding that fecal contamination from young children dominated human fecal contamination on hands is an important finding. Hands have previously been identified as an critical exposure point for bacterial and viral pathogens in Tanzania[39] and child hand-to-mouth contacts were estimated to be responsible for 97% of the fecal contamination (based on *E. coli*) consumed among hand-to-mouth contacts and water consumption.[40] Our study also found children's hands tended to be washed infrequently, with an average of 9.7 hours since the last wash (SD 5.5, range 0.5-23), and that while caregivers reported almost always washing their hands with soap after using the toilet (97% reported doing so), they often did not do so after cleaning a child (33%

reported doing so). Taken together, these results suggest that handwashing interventions that target washing children's hands more frequently and that focus on caregivers washing their hands after cleaning their children may be targeted activities that can reduce hand contamination in this community.

The results that child fecal contamination was found to be dominant more often inside the household than older child/adult feces may also help to explain why other studies have found higher levels of fecal contamination in households than latrines. In a peri-urban area in Tanzania, Pickering et al. found higher levels of fecal indicator bacteria in soil samples from household floors than soil sampled from latrine floors, indicating that other sources contribute to fecal contamination exposure in households.[41] Also in Tanzania, a study in urban and rural areas found that although the amount of fecal contamination was lower on household surfaces than latrine surfaces, there was no correlation between fecal contamination in latrines and on household surfaces or between helminth concentration in soil around latrine and in soil in the household.[5] Young children commonly defecate inside the home, and diapers or potties may also be cleaned inside the home (Figure 3.1), creating potential for young children's feces to contaminate the domestic environment independent of toilet facility access.

The identification of young children's feces contaminating household surfaces also provides evidence explaining links other studies have found between child feces disposal practices and child health. Previously, negative child health consequences including diarrhea, helminth infections, environmental enteric dysfunction, and stunting have each been associated with child faeces disposal practices.[18,19,42,43] Our results provide evidence of precise points of exposure that

children could have to fecal contamination from young children's feces and offer an experimental method that could evaluate the success or failure of sanitation and hygiene interventions targeting child feces management practices for reducing fecal contamination exposure originating from young children's feces.

Broader implications and study limitations

The results of this work suggest two contributions. The first contribution is the validation of a tool to track young children's feces separately from older child/adult feces, which could be a valuable addition to the toolbox for evaluating sanitation interventions and understanding how these interventions affect fecal pathogen transmission pathways. The second contribution is the use of an observation and survey-independent tool to understand the prevalence and distribution of fecal contamination from young children in the domestic environment.

This study has some notable limitations. First, this study only included human fecal sources and did not consider animal sources that may also be important for transmission of fecal pathogens. This study also focused on tracking fecal contamination, but the detection of specific fecal pathogens at exposure points would provide greater ability to link exposure to human health. Additionally, the exposure route of foods consumed was excluded from this study and could potentially be an important exposure route. Furthermore, since ambient samples were collected for this analysis, there is the potential for bacteria components of the contamination sources to degrade or grow at different rates in the environment, which could impact identification by this analysis.[15] Additionally, although community methods have the benefit that they are less susceptible to regional variability than single biomarker methods,[15] more work would be needed

to verify if using sequencing data in existing databases would be sufficient for source tracking of young children's feces separately from other human sources, or if locally collected fecal sample sequencing data would be required. There were also several environmental samples that tested positive for the human-associated HF183 marker that were not identified by SourceTracker to be contaminated with young child fecal or older child/adult fecal sources. This may be due to the cross reactivity of human markers with chicken and dogs which has been demonstrated in another urban slum setting,[44] although it is also possible the sensitivity of microbial community source tracking is lower than single markers.[15]

Despite its limitations, this study provides evidence that young children's feces are an important contributor to household fecal contamination in urban slums and more research is needed on effective methods of child feces management. Thus far, there are limited data on the success of child feces management interventions,[45] with a few studies showing poor improvements in child defecation or feces disposal resulting from interventions [46–48] and one study showing successful outcomes.[49] Improvements in sanitation infrastructure and latrine construction as well as drainage infrastructure and solid waste management have the potential to reduce environmental contamination from adult and child human fecal sources. This study also demonstrates the potential of using microbial community methods for tracking young children's feces separately from other human fecal contamination sources in domestic environments, which could be used to design targeted interventions for reducing fecal contamination as well as to evaluate how sanitation interventions affect fecal pathogen transmission pathways.

3.5 Acknowledgements

The authors thank Phantus Wambiya, Rodah Obondi, and Philip Omandid for their help conducting household interviews and fieldwork, Gabrielle Levato for her help with field sampling and lab work, Professor Robinson Ocharo at the University of Nairobi for assistance organizing fieldwork, and Sanergy for providing fecal samples collected from their urine-diversion toilets. We also thank the participating households who made this study possible. Valerie Bauza was supported by the National Science Foundation Graduate Research Fellowship Program under grant no. DGE-1144245 while conducting this work, and travel and supplies were supported by the Safe Global Water Institute (SGWI) and the Department of Civil and Environmental Engineering at the University of Illinois at Urbana-Champaign.

Table 3.1. Sensitivity and specificity for human fecal contamination source identification.

	Dominant fecal source identified				Presence/absence of source identified			
	Young child		Older child & adult		Young child		Older child & adult	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
V3-V4 region, with soil assigned as a "source"								
All samples	100%	100%	100%	100%	100%	100%	100%	86%
Water samples	100%	100%	100%	100%	100%	100%	100%	80%
Soil samples	100%	100%	100%	100%	100%	100%	100%	100%
V4 region, with soil assigned as a "source"								
All samples	100%	100%	100%	100%	100%	100%	100%	86%
Water samples	100%	100%	100%	100%	100%	100%	100%	80%
Soil samples	100%	100%	100%	100%	100%	100%	100%	100%
V3-V4 region, without soil assigned as a "source"								
All samples	100%	87%	88%	100%	100%	71%	100%	94%
Water samples	100%	91%	92%	100%	100%	70%	100%	100%
Soil samples	100%	75%	75%	100%	100%	75%	75%	100%
V4 region, without soil assigned as a "source"								
All samples	100%	67%	71%	100%	100%	50%	78%	100%
Water samples	100%	91%	92%	100%	100%	70%	100%	100%
Soil samples	100%	0%	0%	100%	100%	0%	0%	100%

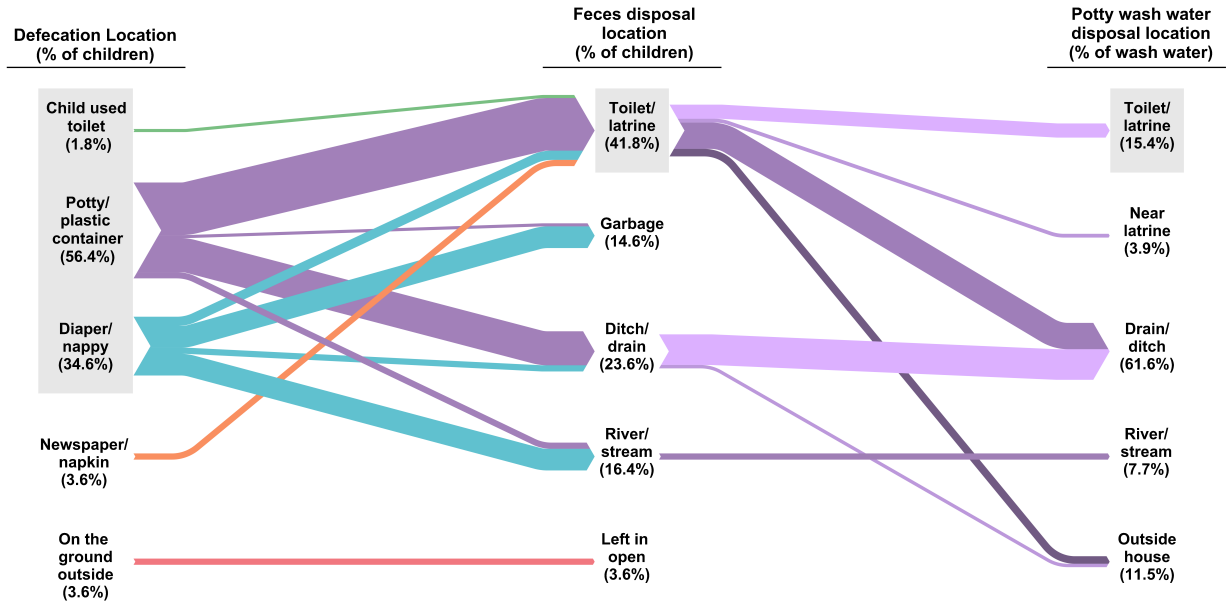


Figure 3.1. Child defecation, feces disposal, and potty wash water disposal locations. Locations that are considered safe for each activity are highlighted in gray boxes. The thickness of the each arrow corresponds to the percentage of children that each combination of practices represents. The potty wash water disposal location is only shown for children that were reported to defecate in a child potty or plastic container (purple arrows).

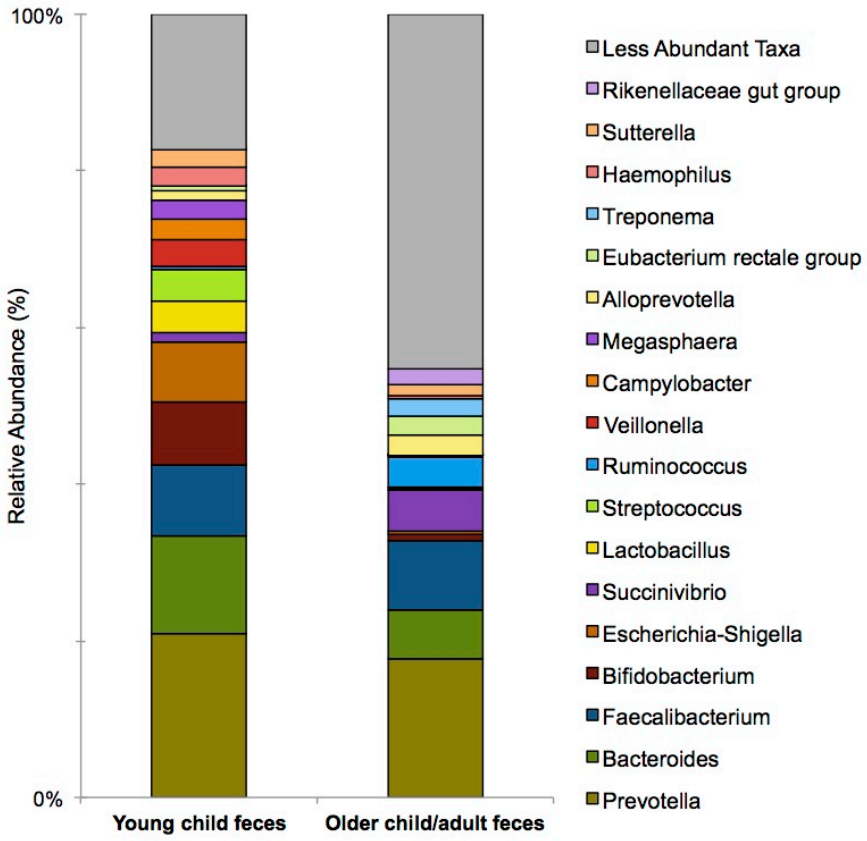


Figure 3.2. Differences in the average relative abundance of genus-level taxa for young child and older child/adult fecal samples using the V3-V4 region of the 16S rRNA gene primer set. The "Less Abundant Taxa" classification includes taxa with less than 2% average relative abundance among samples, which has a greater relative abundance in older child/adult feces than young child feces. Similar results for the V4 region are presented in Figure B.1 in the SI.

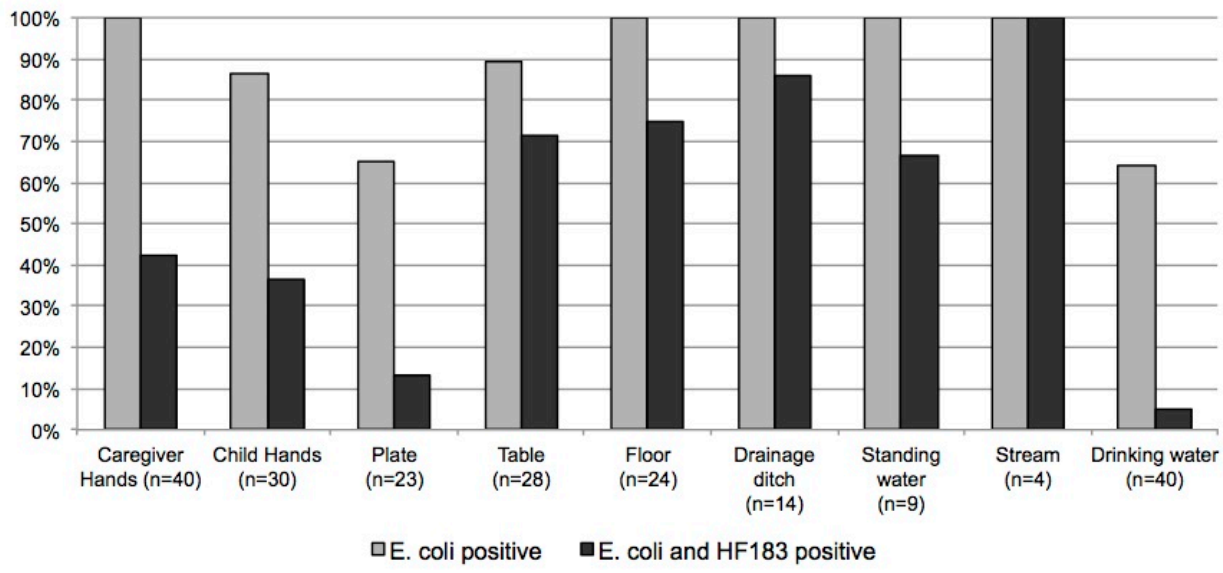


Figure 3.3. The proportion of environmental sample positive for *E. coli* and for both *E. coli* and the human-associated *Bacteroides* fecal marker HF183. The positive detection of the human-associated fecal marker indicates that human fecal contamination of the sample is likely, so samples positive for both *E. coli* and the human-associated fecal marker indicators were sequenced to determine the specific source of human fecal contamination.

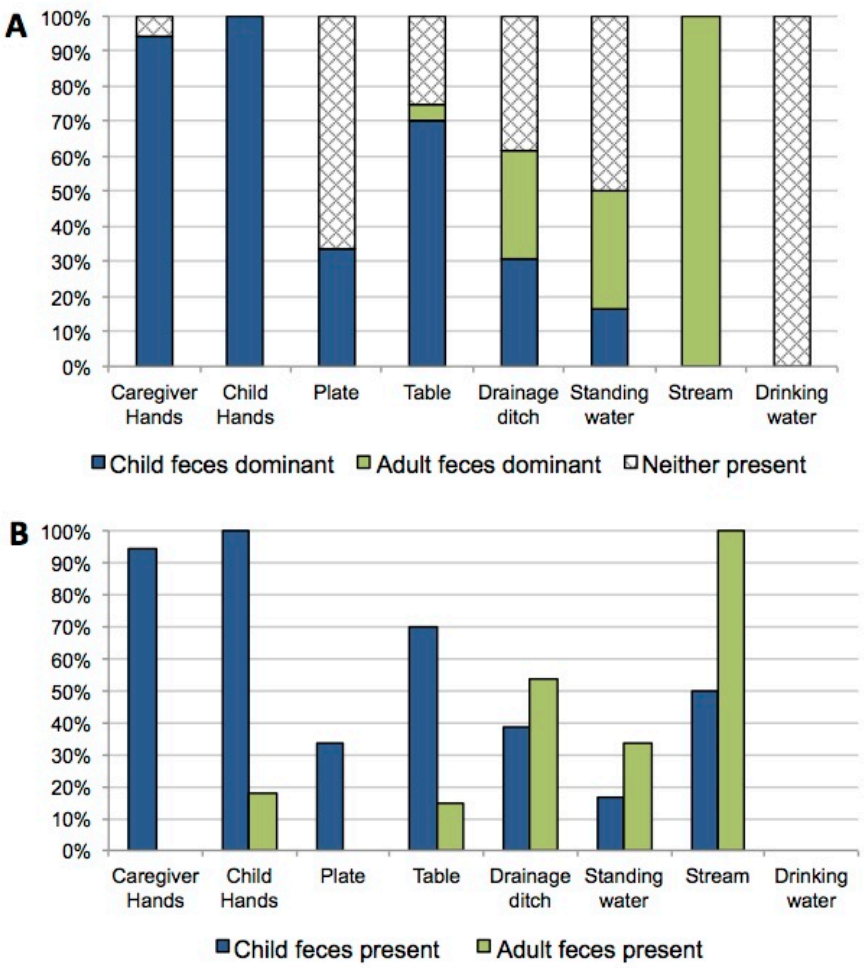


Figure 3.4. Environmental sample results for (A) dominant human-associated source and (B) presence/absence of contamination source among samples that tested positive for the HF183 human fecal marker. Similar results for the V4 region are present in Figure B.3 in the SI.

3.6 References

1. World Health Organization. Diarrhoeal disease fact sheet. *WHO* (2017). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/. (Accessed: 6th June 2017)
2. Mbuya, M. N. & Humphrey, J. H. Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries. *Matern. Child. Nutr.* 12, 106–120 (2016).
3. Black, R. E. *et al.* Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet* 382, 427–451 (2013).
4. Curtis, V., Cairncross, S. & Yonli, R. Review: Domestic hygiene and diarrhoea–pinpointing the problem. *Trop Med Int Health* 5, 26–30 (2000).
5. Exley, J. L. R., Liseka, B., Cumming, O. & Ensink, J. H. J. The Sanitation Ladder, What Constitutes an Improved Form of Sanitation? *Environ. Sci. Technol.* 49, 1086–1094 (2015).
6. Clasen, T. F. *et al.* Interventions to improve disposal of human excreta for preventing diarrhoea. in *Cochrane Database of Systematic Reviews* (ed. The Cochrane Collaboration) (John Wiley & Sons, Ltd, 2010).
7. Brown, J., Cairncross, S. & Ensink, J. H. J. Water, sanitation, hygiene and enteric infections in children. *Arch. Dis. Child.* 98, 629–634 (2013).
8. Hagedorn, C., Blanch, A. R. & Harwood, V. J. *Microbial source tracking: methods, applications, and case studies*. (Springer Science & Business Media, 2011).
9. Boehm, A. B. *et al.* Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. *Water Res.* 47, 6812–6828 (2013).
10. Henry, R. *et al.* Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters. *Water Res.* 93, 242–253 (2016).
11. Unno, T. *et al.* Use of Barcoded Pyrosequencing and Shared OTUs To Determine Sources of Fecal Bacteria in Watersheds. *Environ. Sci. Technol.* 44, 7777–7782 (2010).
12. Cao, Y., Van De Werfhorst, L. C., Sercu, B., Murray, J. L. S. & Holden, P. A. Application of an Integrated Community Analysis Approach for Microbial Source Tracking in a Coastal Creek. *Environ. Sci. Technol.* 45, 7195–7201 (2011).
13. Newton, R. J., Bootsma, M. J., Morrison, H. G., Sogin, M. L. & McLellan, S. L. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. *Microb. Ecol.* 65, 1011–1023 (2013).
14. Dubinsky, E. A. *et al.* Application of Phylogenetic Microarray Analysis to Discriminate Sources of Fecal Pollution. *Environ. Sci. Technol.* 46, 4340–4347 (2012).
15. Cao, Y. *et al.* Evaluation of molecular community analysis methods for discerning fecal sources and human waste. *Water Res.* 47, 6862–6872 (2013).
16. Yatsunenko, T. *et al.* Human gut microbiome viewed across age and geography. *Nature* (2012). doi:10.1038/nature11053
17. Yeager, B. A., Huttly, S. R., Bartolini, R., Rojas, M. & Lanata, C. F. Defecation practices of young children in a Peruvian shanty town. *Soc. Sci. Med.* 49, 531–541 (1999).
18. Gil, A., Lanata, C., Kleinau, E. & Penny, M. Strategic Report 11 Children's Feces Disposal Practices in Developing Countries and Interventions to Prevent Diarrheal Diseases. *US Agency Int. Dev. Wash. DC* 20523, (2004).
19. Bauza, V. & Guest, J. S. The effect of young children's faeces disposal practices on child growth: Evidence from 34 countries. *Trop. Med. Int. Health* (2017).

20. Knights, D. *et al.* Bayesian community-wide culture-independent microbial source tracking. *Nat. Methods* 8, 761–763 (2011).
21. Bauza, V., Ocharo, R. M., Nguyen, T. H. & Guest, J. S. Soil Ingestion Is Associated with Child Diarrhea in an Urban Slum of Nairobi, Kenya. *Am. J. Trop. Med. Hyg.* 96, 569–575 (2017).
22. Jenkins, M. W., Tiwari, S., Lorente, M., Gichaba, C. M. & Wuertz, S. Identifying human and livestock sources of fecal contamination in Kenya with host-specific Bacteroidales assays. *Water Res.* 43, 4956–4966 (2009).
23. Brown, S. P., Ferrer, A., Dalling, J. W. & Heath, K. D. Don't put all your eggs in one basket: a cost-effective and powerful method to optimize primer choice for rRNA environmental community analyses using the Fluidigm Access Array. *Mol. Ecol. Resour.* (2016).
24. Caporaso, J. G. *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624 (2012).
25. Hiergeist, A., Reischl, U., Gessner, A. & others. Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability. *Int. J. Med. Microbiol.* 306, 334–342 (2016).
26. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* btu170 (2014).
27. Zhang, J., Kobert, K., Flouri, T. & Stamatakis, A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30, 614–620 (2014).
28. Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336 (2010).
29. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461 (2010).
30. Schloss, P. D. *et al.* Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* 75, 7537–7541 (2009).
31. Isunju, J. B., Schwartz, K., Schouten, M. A., Johnson, W. P. & van Dijk, M. P. Socio-economic aspects of improved sanitation in slums: a review. *Public Health* 125, 368–376 (2011).
32. Stanton, B. F., Clemens, J. D., Aziz, K. M. A. & Rahman, M. Twenty-four-hour recall, knowledge-attitude-practice questionnaires, and direct observations of sanitary practices: a comparative study. *Bull. World Health Organ.* 65, 217 (1987).
33. Manun'Ebo, M. *et al.* Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. *Trop. Med. Int. Health* 2, 1015–1021 (1997).
34. Wu, G. D. *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334, 105–108 (2011).
35. Pop, M. *et al.* Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. *Genome Biol.* 15, R76 (2014).
36. De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci.* 107, 14691–14696 (2010).
37. Favier, C. F., Vaughan, E. E., De Vos, W. M. & Akkermans, A. D. Molecular monitoring of succession of bacterial communities in human neonates. *Appl. Environ. Microbiol.* 68, 219–226 (2002).

38. Mackie, R. I., Sghir, A. & Gaskins, H. R. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clin. Nutr.* 69, 1035s–1045s (1999).
39. Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J. & Boehm, A. B. Hands and Water as Vectors of Diarrheal Pathogens in Bagamoyo, Tanzania. *Environ. Sci. Technol.* 47, 355–363 (2013).
40. Mattioli, M. C. M., Davis, J. & Boehm, A. B. Hand-to-Mouth Contacts Result in Greater Ingestion of Feces than Dietary Water Consumption in Tanzania: A Quantitative Fecal Exposure Assessment Model. *Environ. Sci. Technol.* 49, 1912–1920 (2015).
41. Pickering, A. J. *et al.* Fecal Contamination and Diarrheal Pathogens on Surfaces and in Soils among Tanzanian Households with and without Improved Sanitation. *Environ. Sci. Technol.* 46, 5736–5743 (2012).
42. Roy, E. *et al.* Patterns and risk factors for helminthiasis in rural children aged under 2 in Bangladesh. *South Afr. J. Child Health* 5, 78–84 (2011).
43. George, C. M. *et al.* Unsafe Child Feces Disposal is Associated with Environmental Enteropathy and Impaired Growth. *J. Pediatr.* (2016).
44. Harris, A. R. *et al.* Ruminants contribute fecal contamination to the urban household environment in Dhaka, Bangladesh. *Environ. Sci. Technol.* 50, 4642–4649 (2016).
45. Morita, T., Godfrey, S. & George, C. M. Systematic review of evidence on the effectiveness of safe child faeces disposal interventions. *Trop. Med. Int. Health* 21, 1403–1419 (2016).
46. Stanton, B. F. & Clemens, J. D. An educational intervention for altering water-sanitation behaviors to reduce childhood diarrhea in urban Bangladesh II. A randomized trial to assess the impact of the intervention on hygienic behaviors and rates of diarrhea. *Am. J. Epidemiol.* 125, 292–301 (1987).
47. Curtis, V. *et al.* Evidence of behaviour change following a hygiene promotion programme in Burkina Faso. *Bull. World Health Organ.* 79, 518–527 (2001).
48. Yeager, B. A. *et al.* An intervention for the promotion of hygienic feces disposal behaviors in a shanty town of Lima, Peru. *Health Educ. Res.* 17, 761–773 (2002).
49. Jinadu, M. K., Adegbenro, C. A., Esmai, A. O., Ojo, A. A. & Oyeleye, B. A. Health promotion intervention for hygienic disposal of children's faeces in a rural area of Nigeria. *Health Educ. J.* 66, 222–228 (2007).

CHAPTER 4: THE EFFECT OF YOUNG CHILDREN'S FECES DISPOSAL PRACTICES ON CHILD GROWTH: EVIDENCE FROM 34 COUNTRIES[‡]

4.1 Abstract

Objective: To characterize the relationship between child faeces disposal and child growth in low- and middle-income countries.

Methods: We analysed caregiver responses and anthropometric data from Demographic and Health Surveys (2005-2014) for 202,614 children under five and 82,949 children under two to examine the association between child faeces disposal and child growth.

Results: Child faeces disposal in an improved toilet was associated with reduced stunting for children under five [adjusted prevalence ratio (aPR)=0.90, 95% confidence interval (CI) 0.89-0.92] and a 0.12 increase in height-for-age z-score (HAZ; 95% CI 0.10-0.15) among all households. Among households with improved sanitation access, practicing improved child faeces disposal was still associated with a decrease in stunting (aPR=0.94, 95% CI 0.91-0.96) and a 0.09 increase in HAZ (95% CI 0.06-0.13). Improved child faeces disposal was also associated with reductions in underweight and wasting, and an increase in weight-for-age z-score (WAZ), but not an increase in weight-for-height z-score (WHZ). Community coverage level of improved child faeces disposal was also associated with stunting, with 75-100% coverage associated with the greatest reduction in stunting. Child faeces disposal in an unimproved toilet was associated with reductions in underweight and wasting, but not stunting.

[‡] This chapter has been published: Bauza, V., & Guest, J. S. (2017). The effect of young children's faeces disposal practices on child growth: Evidence from 34 countries. *Tropical Medicine & International Health*.

Conclusions: Improved child faeces disposal practices could achieve greater reductions in child undernutrition than improving toilet access alone. Additionally, the common classification of child faeces disposal as 'safe' regardless of the type of toilet used for disposal may underestimate the benefits of disposal in an improved toilet and overestimate the benefits of disposal in an unimproved toilet.

4.2 Introduction

More than one-quarter of children under five in developing countries are stunted (1). Stunting and other forms of undernutrition determined by anthropometric measures of child growth (defined in detail in the Methods section) impair immune response, increasing the risk of death from infectious diseases such as diarrhoea and pneumonia (1–3). As a result, 14.7% of all deaths in children under five are attributed to stunting (1). Stunting in childhood may also have long-term consequences, leading to lower cognitive ability, lower adult economic productivity, and increased risk of developing obesity and associated chronic diseases later in life (4).

Several studies have demonstrated a link between water, sanitation, and hygiene (WASH) and child growth. A recent trial in rural Mali observed reduced child stunting in intervention villages that received a community-led sanitation intervention that increased private latrine ownership from 35% to 65% (5). Additionally, analysis of Demographic and Health Surveys (DHS) data for eight countries found that sanitation improvements with or without water supply improvements were associated with increases in linear growth of children (6). Similarly, a cohort study in Peru found linear growth deficits were associated with poor conditions for sanitation, water source, and water storage (7), and a cohort study in Bangladesh found reduced faecal environmental

contamination of households (estimated using surveyed WASH conditions) was associated with increased height-for-age z-scores (8). Community-wide sanitation coverage has also been linked with child growth, and a recent cohort study in rural Ecuador found that community-level sanitation coverage (defined as the proportion of households within 500 metres of a household that had access to improved sanitation) was a much stronger predictor of child stunting than the individual household's access to improved sanitation (9).

Although much evidence links sanitation facilities to child growth, child faeces disposal practices must be evaluated separately from sanitation access improvements, because children are often not able or choose not to use toilet facilities. For example, the recent community-led sanitation intervention trial in Mali found open defecation to be practiced by less than 10% of adults in the intervention area, but more than 40% of children (5). When not using a toilet facility, young children may defecate in various places such as diapers/nappies, child potties, or on the ground inside or outside the home (10). From there, children's faeces may be left in place (inside or outside), buried in the soil, or disposed of in a toilet/latrine, in a ditch or drain, in the yard, in a river or canal, or with garbage (10–13). There have been some discrepancies between classifications of child faeces disposal practices as sanitary among different groups (14), but the Water and Sanitation Program (World Bank Group) and UNICEF have classified 'safe' child faeces disposal as a child using a toilet/latrine or the child's faeces being put in a toilet/latrine (regardless of the type of toilet/latrine) and 'improved' child faeces disposal practices as a child using or his/her faeces being put into an improved toilet/latrine (15). Throughout this paper, we will also use the term 'unimproved' faeces disposal to refer a child using or his/her faeces being

put into an unimproved toilet/latrine and the term 'unhygienic' faeces disposal to refer to practices that do not fall into unimproved, safe, or improved classifications.

Unhygienic child faeces disposal is not uncommon in households with improved sanitation (16), which is concerning because exposure to children's faeces may present a greater health risk than exposure to adult faeces. Children tend to have a higher prevalence of diarrheal disease and soil-transmitted helminth infections, and thus their faeces may contain higher levels of pathogens and helminth eggs (17). A meta-analysis of six studies found that improper handling or disposal of young children's faeces was associated with a 23% increased risk of diarrhoea [risk ratio (RR)=1.23, 95% confidence interval (CI) 1.15-1.32] (12). Additionally, one study in rural Bangladesh found that disposal of child faeces in closed spaces (as compared to open spaces) resulted in a 35% reduction of helminth infections (18). Although an association between unsafe child faeces disposal and impaired growth was also found in a small cohort in rural Bangladesh (19), the link between child faeces disposal practices and child growth has not been comprehensively investigated or assessed on a large-scale. Additionally, differential effects of disposal of child faeces into unimproved versus improved toilet facilities has not been previously investigated, and past studies have often combined these categories together which may overestimate the positive health effects associated with disposal into unimproved facilities.

The objective of this study is to evaluate the association between child faeces disposal practices and child growth. DHS observations from 34 low- and middle-income countries were used to evaluate this link for household child faeces disposal practices and community-coverage levels of improved child faeces disposal. We evaluated the associations between child faeces disposal

and child growth among all households with children to evaluate the maximum benefits of these practices as well as among households with children that already had access to improved sanitation to elucidate the incremental benefits of improved child faeces management after improved toilet installation. As a secondary objective, we also assessed the difference between associations with child growth from disposal of child faeces into unimproved toilets compared to improved toilets to evaluate if the common practice of classifying both of these practices together as safe disposal should be reconsidered. This study focused on low- and middle-income countries because these countries tend to have a high burden of disease attributed to child undernutrition (20).

4.3 Methods

Data source and study population

This study uses cross-sectional household survey data collected by DHS, which are described in detail elsewhere (21). The surveys use a two-stage sampling process, which provides representative samples for each country as well as urban and rural areas. Each survey was administered to the female household caregiver and included several questions pertaining to household demographics, educational attainment of household caregivers, water and sanitation practices, and child health. The height and weight of children were also measured.

The focus of this study was developing countries, and therefore DHS surveys for countries classified as developed were excluded. Countries classified as small island developing states were also excluded due to their unique water and sanitation challenges that may not be generalizable to the larger sample frame. All remaining surveys were considered for inclusion.

Countries were further excluded if insufficient information was collected pertaining to child faeces disposal practices, household toilet facility, or child height and weight. In total, surveys from 34 countries representing 202,614 children under five were used for this analysis. All included surveys were conducted from between 2005 and 2014 and the most recent survey data available for each country that contained sufficient information on the exposure and outcome variables were used.

Variables

Several anthropometric measures of child growth were analysed as outcome variables. The primary outcome variable of interest was stunting. A child was defined as stunted if he/she had a height-for-age z-score (HAZ) less than -2, meaning that his/her height was more than two standard deviations below the international reference height for a child of that age. Other outcome variables used in the analysis include the continuous variables of HAZ, weight-for-age z-score (WAZ), and weight-for-height z-score (WHZ), and dichotomous variables of severe stunting (HAZ<-3), underweight (WAZ<-2), severely underweight (WAZ<-3), wasting (WHZ <-2), and severe wasting (WHZ <-3). All z-scores were calculated using current World Health Organization guidelines (22). Throughout this paper, we use the terms child growth to refer to any of these anthropometric measures related to child height or weight, linear growth to refer to anthropometric measures related to height, and undernutrition to refer to the anthropometric measures of stunting, wasting, and underweight.

The primary independent variable was household child faeces disposal. Each respondent reported how the stools were disposed of the last time their youngest child passed stools. We classified

disposal behaviour as improved for all children in the household if the final disposal location for the household's youngest child's faeces was a toilet/latrine and the household had access to an improved toilet/latrine (defined below). We classified household faeces disposal behaviour as unimproved if the youngest child always uses an unimproved toilet/latrine or if the stool was put or rinsed into an unimproved toilet/latrine. We classified all other disposal behaviours (stools put/rinsed into a drain or ditch, thrown into garbage, buried, or left in open and not disposed of) as unhygienic. Burial and disposal with garbage were classified as unhygienic based on recommendations of a recent expert consultation (14). Children from households reporting that the youngest child used washable or disposable diapers (0.2% of the children in pooled surveys) were excluded from our analysis since information regarding the final disposal of the faeces in the diapers was not reported. Children from households reporting an 'other' disposal method (2.1% of the children in pooled surveys) were also excluded. Community coverage levels for households practicing improved child faeces disposal were calculated as non-self means using primary sampling units as the community level (which we are assuming to correspond to a village-level coverage in rural areas and neighbourhood-level coverage in urban areas). Only households with children were included in this community coverage calculation.

We used the Joint Monitoring Program (JMP) guidelines to classify toilet facilities as improved if the facility was a flush/pour flush toilet to a piped sewer system, septic tank, or pit latrine, was a VIP latrine, a pit latrine with a slab, or a composting toilet (23). However, as our goal was to assess if the toilet facility created a safe barrier to separate human excreta from the environment, toilet facilities were classified as improved regardless of whether or not they were shared with other households. We classified the following facilities as an unimproved toilet: flush/pour flush

toilet to somewhere else, pit latrine without a slab, bucket toilet, and hanging toilet/latrine. We also calculated community coverage levels of improved toilets for households with children as non-self means using primary sampling units to compare with results for improved child faeces disposal coverage.

The following additional independent variables were included in our model as potential confounding variables based on literature (6,24,25): access to an improved water source as defined by JMP (23), international wealth index (IWI), mother's educational attainment, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card. The IWI is an index of household economic wealth that is based on asset ownership and can be used for wealth comparison among low- and middle-income countries (Section C.1 of Supplementary Information [SI]) (26). For children that diet information was collected for, a supplementary analysis was also performed including a dietary diversity indicator score which estimates each child's diet quality based on the diversity of foods consumed in the previous 24 hours (25) as a confounding variable (Section C.1, SI).

Statistical analysis

We used Poisson regression to estimate the prevalence ratio (PR) of stunting, wasting, and underweight associated with household child faeces disposal practices in children under five and under two years of age. We also used linear regression to estimate the association between continuous variables HAZ, WAZ, and WHZ and household child faeces disposal practices. We accounted for complex sampling strategy in all regression models and included fixed effects for

countries in each regression model that pooled countries together. Models were adjusted for potential confounders as detailed above. The characteristics of models used for household-level analysis are shown in Table 4.1. Model 1 characteristics were used to calculate the association of improved child faeces disposal with child growth among all households to evaluate the maximum benefit of this practice. Model 2 characteristics were used to remove any child growth associations that may be due to improved toilet access in Model 1 results by only including households with access to improved sanitation in the calculations. Model 3 characteristics were used to assess the differences in child growth associations for improved disposal compared to unimproved disposal. We calculated the association between child growth and child faeces disposal in the pooled sample, and data stratified by residence area (urban/rural), geographic region [Sub-Saharan Africa (SSA), and Asia and North Africa (ANA)], and country. The regional grouping used to track the United Nation's Millennium Development Goals (MDG) progress were used for this study. However, due to the small number of included surveys in the South Asia, Southeastern Asia, Caucasus and Central Asia, and North Africa regions, these regions were combined into a single Asia and North Africa region for analysis. Additionally, Madagascar was included in the pooled analysis but excluded from country specific analysis to avoid sparse data bias as less than 2.5% of the population used improved child faeces disposal. We used Stata version 13.1 (StataCorp LP, College Station, TX, USA) for all analyses.

4.4 Results

All households with all types of sanitation access (Model 1 characteristics)

In the pooled dataset, an average of 36.8% of children were stunted, 11.0% were wasted, 21.7% were underweight, 45.2% had access to an improved toilet facility, 21.8% had access to a

unimproved toilet/latrine, 31.6% were in households that participated in improved child faeces disposal, and 17.8% were in households that participated in unimproved disposal of child faeces. However, household toilet access, household faeces disposal practices, and the prevalence of stunting and other anthropometric outcomes for children under five varied substantially by regional area (Table 4.2) and country (Table C.1 in SI).

Improved child faeces disposal practices were associated with reductions in stunting, severe stunting, underweight, severely underweight, and wasting, and increases in HAZ and WAZ for pooled data including all households (Tables 4.3 and 4.4; Model 1 characteristics). (See Tables C.2 and C.3 in SI for unadjusted results.) In the pooled sample, use of improved child faeces disposal practices was associated with a 10% reduction in the prevalence of stunting in children under five (aPR=0.90, 95% CI 0.89-0.92) and a 13% reduction in the prevalence of severe stunting (aPR=0.87, 95% CI 0.84-0.90) (Table 4.3). Use of improved child faeces disposal practices was also associated with a 0.12 increase in HAZ (95% CI 0.10-0.15) (Table 4.4). Similar trends and associations were observed in the underweight, severely underweight, wasting and WAZ data and no associations were observed in the severe wasting or WHZ data (Tables 4.3 and 4.4). Additionally, the magnitude and trends of adjusted models for children under two were generally similar to results for children under five (Tables 4.3 and 4.4). A summary of conclusions across different household-level models is provided in Table 4.5.

When stratified by geographic region, improved child faeces disposal practices were associated with reductions in stunting and severe stunting and increases in HAZ in SSA and ANA, with stronger associations seen in ANA (SSA: aPR=0.92, 95%CI 0.90-0.94, severe stunting

aPR=0.91, 95%CI 0.88-0.95, HAZ increase=0.09, 95% CI 0.07-0.12; ANA: stunting aPR=0.88, 95% CI 0.85-0.90, severe stunting aPR=0.79, 95%CI 0.74-0.83, HAZ increase=0.16, 95% CI 0.12-0.20). Similar trends and associations were observed in the underweight, severely underweight, and WAZ data, an association with wasting was observed for ANA but not SSA, and no associations were observed in the severe wasting or WHZ data (Tables 4.3 and 4.4). When stratified by residence area, the associations between improved child faeces disposal and improved anthropometric outcomes did not substantially vary between urban areas and rural areas for most anthropometric outcomes (Tables C.5 and C.6 in SI).

There was heterogeneity in the results across countries, although the majority (88%) of countries had adjusted prevalence ratios less than one for stunting, indicating that improved child faeces disposal was associated with reduced stunting (Figure 4.1). Additionally, 91% of countries had HAZ coefficients greater than zero, indicating that improved child faeces disposal practices were associated with increased HAZ (Figure C.2, SI). However, although a large majority of countries had aPRs for stunting that were less than one and HAZ coefficients greater than zero, it should be noted that only 30% of the aPRs for stunting and 48% of the HAZ coefficients were statistically significant. (See Figures C.1 and C.2 in SI for country-specific results of other anthropometric outcomes.)

Households with improved sanitation access (Model 2 characteristics)

Among households with access to improved toilets, improved child faeces disposal practices remained associated with a decrease in stunting, severe stunting, underweight, and severely

underweight (Figure 4.2, Table 4.3) and an increase in HAZ and WAZ (Table 4.4) for children under five.

All households: improved vs. unimproved child faeces disposal (Model 3 characteristics)

When improved child faeces disposal practices and unimproved child faeces disposal practices were included in the same model (Model 3 characteristics), improved child faeces disposal practices remained strongly associated with reductions in stunting and severe stunting, but unimproved child faeces disposal practices were not associated with either (Figure 4.3). Among children under five, both improved and unimproved disposal were associated with reductions in underweight, severely underweight, and wasted outcomes, but improved disposal was associated with greater reductions in underweight and severely underweight outcomes than unimproved disposal (Figure 4.3). (See Table C.4 in SI for HAZ, WAZ, and WHZ results.) However, if safe disposal was instead modelled as a separate variable including both improved and unimproved disposal (a common classification used in other studies), it would be associated with reduced stunting (aPR=0.96, 95%CI 0.94-0.97) and severe stunting (aPR=0.94, 95%CI 0.91-0.96) outcomes that did not have associations with unimproved disposal.

All households: community coverage levels

Community coverage level of improved child faeces disposal practices was also associated with reduced stunting, with the largest reduction in stunting occurring when coverage was over 75% (Table 4.6). The trend was similar to that seen for community coverage of improved toilets among households with children, but the association was stronger for coverage of improved child faeces disposal (Figure 4.4). When a variable for improved child faeces disposal at the household

level and interaction variables between household and community coverage were added to the model, household and community coverage terms remained significant and the interaction terms were not significant, with the exception of 50-75% coverage (Table 4.6). This indicates that household and community coverage levels may both be independently associated with prevalence of stunting. When percent coverage was modelled as a continuous variable, each percentage increase in coverage was associated with a 0.0020 increase in HAZ (95% CI 0.0016-0.0024), although the results of the Poisson regression indicate that a linear interpretation of these data across 0 to 100% coverage would not be appropriate. When broken into 25% coverage intervals, each percentage increase in coverage was associated with a 0.0049 increase in HAZ (95% CI 0.0020-0.0078) among 75-100% coverage, but there were no strong associations for the other coverage intervals (regression coefficients estimating the HAZ increase associated with each percentage increase in coverage were 0.0002 (95% CI -0.0017-0.0022) among 0-25% coverage, -0.0020 (95% CI -0.0056-0.0015) among 25-50% coverage, and 0.0012 (95% CI -0.0020-0.0045) among 50-75% coverage.

4.5 Discussion

This study found a strong association between household child faeces disposal practices and child growth, with improved child faeces disposal practices being associated with reduced levels of child stunting and underweight and increases in HAZ and WAZ. The results indicate that toilet installation is insufficient on its own for eliminating child stunting due to inadequate sanitation practices, and emphasize the importance of promoting hygienic child faeces disposal practices in communities. We also found preliminary evidence that community coverage levels of improved child faeces disposal practices are associated with reduced stunting and increased

HAZ, with the strongest protective effect for community coverage of 75-100%, highlighting the importance of community-level child faeces management.

To our knowledge, our study is the first to provide evidence for a link between child faeces disposal and stunting. When child faeces management practices do not create an adequate barrier between child faeces and the environment, then the child who defecated, other children in the same household, and children in neighbouring households could be exposed to pathogens from the faeces. Acute exposure to faecal pathogens can cause diarrhoea and helminth infections, and repeated exposure to faecal contamination is believed to cause environmental enteric dysfunction – a subclinical condition which causes functional and structural changes to occur in the small intestine that lead to decreased nutrient absorption, increased intestinal permeability, and impaired immune function (29,30). Repeated diarrhoea episodes and intestinal worm infections have each been linked with growth stunting in children (27,28) and there is increasing evidence that environmental enteric dysfunction is also linked with impaired growth (31,32). Additionally, diarrhoea, helminth infections, and environmental enteric dysfunction have each been shown to be associated with child faeces disposal practices (12,18,19) and are likely explanations of the link between child faeces disposal and child growth in this study. However, the new link our study demonstrates between child faeces disposal and child stunting could have important health implications, as stunting and other forms of undernutrition are believed to increase a child's susceptibility to infections as well as increase the risk of a child dying from common infections, such as diarrhoea, pneumonia, and measles (1–3). Thus, reductions in undernutrition could lead to reductions in related child morbidity and mortality. Prior to our work, a recent cohort study in rural Bangladesh with 216 children aged 6 to 30 months found an association between unsafe

child faeces disposal and child wasting, reduced WAZ and WHZ, but no association with reduced stunting (19). While this supports our findings that child faeces disposal practices are associated with WAZ, we did not find a consistent association between faeces disposal practices and WHZ and only found an association with wasting in some of our models, suggesting these short-term nutritional status indicators may be more strongly influenced by site-specific factors, whereas outcomes associated with long-term nutrition indicators like stunting may be more widely representative across populations.

Our study is also the first to evaluate the effect of household child faeces disposal in an unimproved toilet compared to improved toilet on child growth. Our results show that while improved child faeces disposal was associated with a reduction in child stunting and increase in HAZ, unimproved child faeces disposal practices were not. Unimproved child faeces disposal was associated with reductions in underweight and wasting in children under five, although the reduction in underweight was smaller than that for improved disposal and there were no significant associations between unimproved child faeces disposal and improved anthropometric outcomes for children under two. These finding suggest that an improved toilet can provide a better barrier between child faeces and the environment than an unimproved toilet, and that this improvement could have positive health outcomes for children. Our results also highlight how classification of child faeces disposal practice can influence results, as safe child faeces disposal practices – disposal in an unimproved or improved toilet, as defined by the Water and Sanitation Program (World Bank Group) and UNICEF – were associated with a decrease in stunting. Therefore, classifying child faeces disposal in any toilet as safe, regardless of the type of toilet used for disposal, may overestimate the benefit of disposal in unimproved toilets and

underestimate the benefit of disposal in improved toilets. We recommend classifications of improved, unimproved, and unhygienic disposal be used in future research and monitoring activities instead of safe and unsafe disposal.

Community coverage level of improved child faeces disposal practices was associated with reduced stunting and increased HAZ, independent of child faeces practices in the child's own household. We also found the association to be stronger once coverage reached 75%. This finding is consistent with findings of previous studies that sanitation coverage is associated with child growth, and that sanitation may provide an increased level of herd protection once a certain coverage level is reached in a community (9,33). A previous study using data from urban Bangladesh also found that community-level coverage of improved latrines in households with children was more strongly associated with increases in child WHZ than household access to an improved latrine, leading to the hypothesis that improved child faeces disposal contributes to reductions in wasting (34). Our study, leveraging data from across 34 countries on child faeces disposal and child growth, supports this finding and goes further, demonstrating benefits of reduced stunting and increased HAZ. Our results also indicate that both household practices and community-level coverage are independently associated with stunting, demonstrating that both large and small-scale promotion of improved child faeces disposal practices may be beneficial.

Although the use of a large dataset with nationally representative data from 34 countries is a strength of our study, there are also notable limitations. First, a causal relationship between child faeces disposal practices and child growth could not be determined due to the cross-sectional nature of this study. Additionally, although our adjusted models include many potential

confounding variables, there may be other confounding factors that were not included in the analysis that could influence the results. Furthermore, only the final disposal method of child faeces is known for this dataset and no information was provided about the defecation location, the amount of time between defecation and final disposal, whether a tool or hands were used if faeces was moved from the defecation location to the disposal location, how the container used for defecation or the ground were cleaned, or handwashing practices after faeces handling. Faeces may not be immediately picked up by the caregiver after defecation and there is potential for faecal contamination to enter the environment through the entire chain of events related to child faeces management, so these additional details would be helpful in assessing potential health risks associated with specific child faeces disposal practices. Future surveys should incorporate more questions related to these practices. Our data also only included the faeces disposal location for the youngest child in the household, which we have used to represent the household child faeces disposal practices. However, households that report hygienic disposal methods for the youngest child could practice unhygienic disposal methods for older children, which would not be captured in our analysis. Additionally, information about solid waste infrastructure was unknown and classifying disposal with garbage as an unhygienic disposal method may have decreased the magnitude of effects measured in our study. Furthermore, although more than three-quarters of country datasets were collected in 2010 or later, other country datasets were collected as early 2005. As a result, some of the data reported, such as prevalence of child health outcomes and sanitation practices may be out of date. However, we expect our main conclusions supporting a link between improved child faeces disposal practices and child growth are still relevant as these conclusions were also evident in recent country datasets collected in 2014. While there were similar trends in results for many of the countries

included in our study, we would caution against applying our results to countries located in other regions of the world not included in this study, as cultural differences may affect child faeces disposal practices. A final limitation is that reporting bias is likely to over-report hygienic behaviour. Past observational studies have observed that more caregivers report putting child faeces in a toilet/latrine than those actually observed doing so during structured observation (35,36). However, this is likely to bias the results towards the null, which would reduce the association between child faeces disposal practices and child growth, and if present, the true association between child faeces disposal and child growth would be stronger than reported in our study.

Educational and behaviour change interventions have been tested for improving child defecation and faeces disposal practices and, although they can be successful (37), many have failed to show sustained improvement in child defecation or faeces disposal practices (38–40). Recent work suggests that reusable diapers and child potties may be helpful for promoting safe child faeces management (41) as well as improving local tools for picking up child faeces (11), however, there is not yet evidence from completed trials regarding the success of these enabling products. Including the promotion of improved child faeces management practices in interventions and national programs to install improved toilets may improve child nutritional status and health benefits from sanitation promotion, and more research is needed to evaluate the most effective methods to promote hygienic child faeces management practices in ways that will achieve sustained uptake.

4.6 Acknowledgements

The authors thank the Demographic and Health Surveys (DHS) Program for providing access to the data used for this study. V.B. was supported by the National Science Foundation Graduate Research Fellowship [grant no. DGE-1144245] and a Carver Fellowship in Engineering at the University of Illinois at Urbana-Champaign.

Table 4.1. Comparison of model characteristics for household-level models.

	Model 1	Model 2	Model 3
Households with children included	All	Households with improved sanitation	All
Independent variables included			
Improved child faeces disposal (vs. unimproved or unhygienic disposal)	Included	-	-
Improved child faeces disposal (vs. unhygienic disposal)	-	Included	Included
Unimproved child faeces disposal (vs. unhygienic disposal)	-	-	Included
Other potential explanatory variables: household access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card	Included	Included	Included

Improved child faeces disposal: child used or faeces put in an improved toilet; Unimproved disposal: child used or faeces put in an unimproved toilet; Unhygienic disposal: faeces left in open, buried, put/rinsed in a drain or ditch, or thrown in garbage.

Table 4.2. Characteristics of children included in analysis.

		Prevalence of undernutrition outcome (%)						Toilet facility[a] (%)			Child faeces disposal[b] (%)		
	Sample Size	Stunting	Severe stunting	Underweight	Severe underweight	Wasting	Severe wasting	No facility	Unimproved	Improved	Unhygienic	Unimproved	Improved
Child age													
Under 5	202,614	36.8	16.8	21.7	6.9	11.0	4.0	33.0	21.8	45.2	50.6	17.8	31.6
Under 2	82,949	29.4	13.3	19.2	6.4	14.2	5.3	32.7	22.0	45.3	54.6	17.0	28.3
Residence area (children under 5)													
Urban	61,246	29.1	12.2	16.5	4.7	9.9	3.7	11.0	15.8	73.2	32.6	13.3	54.2
Rural	141,368	40.2	18.9	24.0	7.8	11.5	4.1	42.5	24.4	33.1	58.3	19.8	21.9
Geographic region (children under 5)													
Sub-Saharan Africa	136,298	37.5	17.4	19.5	5.9	9.4	3.5	34.3	29.3	36.4	46.7	24.7	28.6
Asia and North Africa	66,316	35.4	15.7	26.0	8.7	14.1	5.0	30.4	6.3	63.3	58.5	3.7	37.9

[a] Improved toilet: flush/pour flush toilet to a piped sewer system, septic tank, or pit latrine, VIP latrine, pit latrine with a slab, or composting toilet; Unimproved toilet: flush/pour flush toilet to somewhere else, pit latrine without a slab, bucket toilet, or hanging toilet/latrine.

[b] Improved child faeces disposal: child used or faeces put in an improved toilet; Unimproved disposal: child used or faeces put in an unimproved toilet; Unhygienic disposal: faeces left in open, buried, put/rinsed in a drain or ditch, or thrown in garbage.

Table 4.3. Adjusted prevalence ratios and 95% confidence intervals for stunting, severe stunting, underweight, severe underweight, wasting, and severe wasting due to improved child faeces disposal practices.

	Model 1: All children included in model			Model 2: Only children in households with improved sanitation included in model		
	aPR	95% CI	p-value	aPR	95% CI	p-value
Stunting						
Children under 5	0.90	0.89 – 0.92	<0.001	0.94	0.91 – 0.96	<0.001
Children under 2	0.90	0.88 – 0.93	<0.001	0.93	0.89 – 0.97	0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.92	0.90 – 0.94	<0.001	0.97	0.94 – 1.00	0.086
Asia & North Africa	0.88	0.85 – 0.90	<0.001	0.91	0.88 – 0.95	<0.001
Severe Stunting						
Children under 5	0.87	0.84 – 0.90	<0.001	0.89	0.86 – 0.93	<0.001
Children under 2	0.93	0.88 – 0.98	0.004	0.94	0.88 – 1.01	0.103
Geographic area (children under 5)						
Sub-Saharan Africa	0.91	0.88 – 0.95	<0.001	0.97	0.92 – 1.03	0.318
Asia & North Africa	0.79	0.74 – 0.83	<0.001	0.83	0.78 – 0.89	<0.001
Underweight						
Children under 5	0.87	0.85 – 0.89	<0.001	0.92	0.89 – 0.95	<0.001
Children under 2	0.86	0.82 – 0.90	<0.001	0.90	0.85 – 0.95	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.91	0.88 – 0.94	<0.001	0.95	0.90 – 1.00	0.066
Asia & North Africa	0.81	0.78 – 0.85	<0.001	0.88	0.84 – 0.92	<0.001
Severely Underweight						
Children under 5	0.84	0.80 – 0.89	<0.001	0.91	0.84 – 0.98	0.011
Children under 2	0.87	0.80 – 0.94	0.001	0.92	0.83 – 1.03	0.167
Geographic area (children under 5)						
Sub-Saharan Africa	0.89	0.83 – 0.95	0.001	0.94	0.85 – 1.05	0.293
Asia & North Africa	0.75	0.69 – 0.82	<0.001	0.86	0.78 – 0.95	0.004
Wasting						
Children under 5	0.96	0.92 – 0.999	0.043	1.02	0.97 – 1.08	0.440
Children under 2	0.94	0.90 – 0.99	0.017	1.02	0.96 – 1.10	0.489
Geographic area (children under 5)						
Sub-Saharan Africa	0.97	0.92 – 1.02	0.242	0.97	0.89 – 1.05	0.456
Asia & North Africa	0.93	0.87 – 0.99	0.015	1.03	0.96– 1.10	0.471
Severe Wasting						
Children under 5	0.98	0.92 – 1.05	0.610	1.02	0.94 – 1.12	0.586
Children under 2	1.00	0.92 – 1.09	0.922	1.06	0.95 – 1.19	0.301
Geographic area (children under 5)						
Sub-Saharan Africa	0.98	0.90 – 1.07	0.697	0.98	0.86 – 1.12	0.781
Asia & North Africa	0.98	0.88 – 1.08	0.644	1.02	0.91– 1.15	0.688

aPR = adjusted prevalence ratio; CI = confidence interval

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects. Unadjusted PRs can be found in Table C.2 of the SI.

Table 4.4. Adjusted regression coefficients and 95% confidence intervals for height-for-age, weight-for-age, and weight-for-height z-scores due to improved child faeces disposal practices.

	Model 1: All children included in model			Model 2: Only children in households with improved sanitation included in model		
	Coef.	95% CI	p-value	Coef.	95% CI	p-value
Height-for-age z score						
Children under 5	0.12	0.10 – 0.15	<0.001	0.09	0.06 – 0.13	<0.001
Children under 2	0.13	0.10 – 0.17	<0.001	0.12	0.07 – 0.16	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.09	0.07 – 0.12	<0.001	0.02	-0.02 – 0.07	0.259
Asia & North Africa	0.16	0.12 – 0.20	<0.001	0.14	0.09 – 0.18	<0.001
Weight-for-age z score						
Children under 5	0.08	0.07 – 0.10	<0.001	0.05	0.03 – 0.07	<0.001
Children under 2	0.09	0.06 – 0.11	<0.001	0.05	0.02 – 0.08	0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.05	0.03 – 0.07	<0.001	0.01	-0.02 – 0.05	0.434
Asia & North Africa	0.12	0.10 – 0.15	<0.001	0.07	0.04 – 0.10	<0.001
Weight-for-height z score						
Children under 5	0.002	-0.02 – 0.02	0.851	-0.02	-0.05 – 0.003	0.083
Children under 2	0.02	-0.01 – 0.05	0.142	-0.02	-0.06 – 0.02	0.351
Geographic area (children under 5)						
Sub-Saharan Africa	-0.01	-0.03 – 0.01	0.425	-0.02	-0.06 – 0.02	0.251
Asia & North Africa	0.03	0.00 – 0.07	0.074	-0.02	-0.06 – 0.01	0.214

aPR = adjusted prevalence ratio; CI = confidence interval

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects. Unadjusted regression coefficients can be found in Table C.3 of the SI.

Table 4.5. Comparison of model conclusions for household-level models. Check marks indicate improvements in anthropometric outcomes that were significantly associated ($p<0.05$) with child faeces disposal practices for each model.

	Model 1		Model 2		Model 3			
	Improved child faeces disposal						Unimproved child faeces disposal	
Child age under:	5	2	5	2	5	2	5	2
Stunting	✔	✔	✔	✔	✔	✔		
Severe stunting	✔	✔	✔		✔	✔		
Underweight	✔	✔	✔	✔	✔	✔	✔	
Severe underweight	✔	✔	✔		✔	✔	✔	
Wasting	✔	✔			✔	✔	✔	
Severe wasting								
HAZ	✔	✔	✔	✔	✔	✔		
WAZ	✔	✔	✔	✔	✔	✔	✔	
WHZ							✔	
Related tables and figures	Figures 4.1 and 4.2, Tables 4.3 and 4.4		Figure 4.2, Tables 4.3 and 4.4		Figure 4.3			

Table 4.6. Adjusted prevalence ratios and 95% confidence levels for the association of community coverage levels with stunting.

	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9
Community coverage of improved toilets						
25-50% vs. 0-25% coverage	0.97 (0.95-0.99)	0.98 (0.96-1.00)	0.97 (0.94-0.99)	-	-	-
50-75% vs. 0-25% coverage	0.94 (0.92-0.96)	0.95 (0.93-0.98)	0.96 (0.93-0.99)	-	-	-
75-100% vs. 0-25% coverage	0.88 (0.86-0.90)	0.90 (0.88-0.92)	0.94 (0.91-0.98)	-	-	-
Household improved toilet access (vs. unimproved or open defecation)	-	0.96 (0.94-0.98)	0.96 (0.93-0.996)	-	-	-
Improved toilet interaction terms						
Household improved toilet x 25-50% Community coverage	-	-	1.04 (0.99-1.09)	-	-	-
Household improved toilet x 50-75% Community coverage	-	-	0.99 (0.94-1.04)	-	-	-
Household improved toilet x 75-100% Community coverage	-	-	0.94 (0.90-0.99)	-	-	-
Community coverage of improved child faeces disposal						
25-50% vs. 0-25% coverage	-	-	-	0.95 (0.93-0.97)	0.96 (0.94-0.98)	0.96 (0.94-0.98)
50-75% vs. 0-25% coverage	-	-	-	0.93 (0.91-0.95)	0.95 (0.93-0.98)	0.97 (0.94-1.00)
75-100% vs. 0-25% coverage	-	-	-	0.85 (0.83-0.87)	0.88 (0.86-0.91)	0.89 (0.86-0.93)
Household improved child faeces disposal (vs. unimproved or unhygienic)	-	-	-	-	0.94 (0.92-0.95)	0.96 (0.93-0.99)
Improved child faeces disposal interaction terms						
Household disposal x 25-50% Community coverage	-	-	-	-	-	1.00 (0.95-1.05)
Household disposal x 50-75% Community coverage	-	-	-	-	-	0.95 (0.91-0.998)
Household disposal x 75-100% Community coverage	-	-	-	-	-	0.97 (0.91-1.02)

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects. aPRs for underweight and wasted outcomes can be found in Table C.7 of the SI.

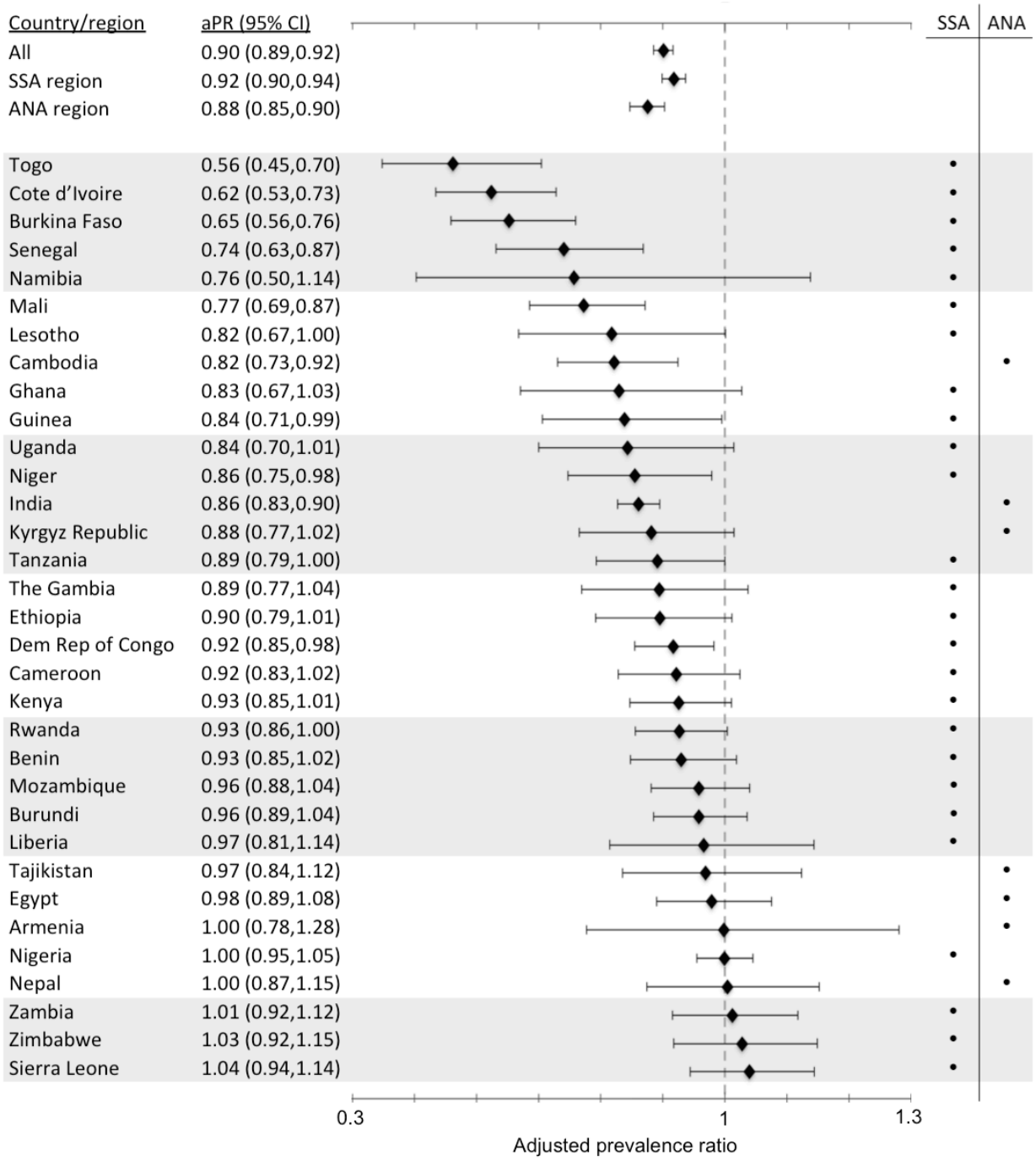


Figure 4.1. Country-specific adjusted prevalence ratio (aPR) and 95% confidence intervals for stunting due to improved child faeces disposal practices for Model 1 characteristics, which includes all pooled households. Models have been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card. Similar graphs for other undernutrition outcomes (underweight, wasting, and z-scores) and for Model 2 characteristics are available in the SI.

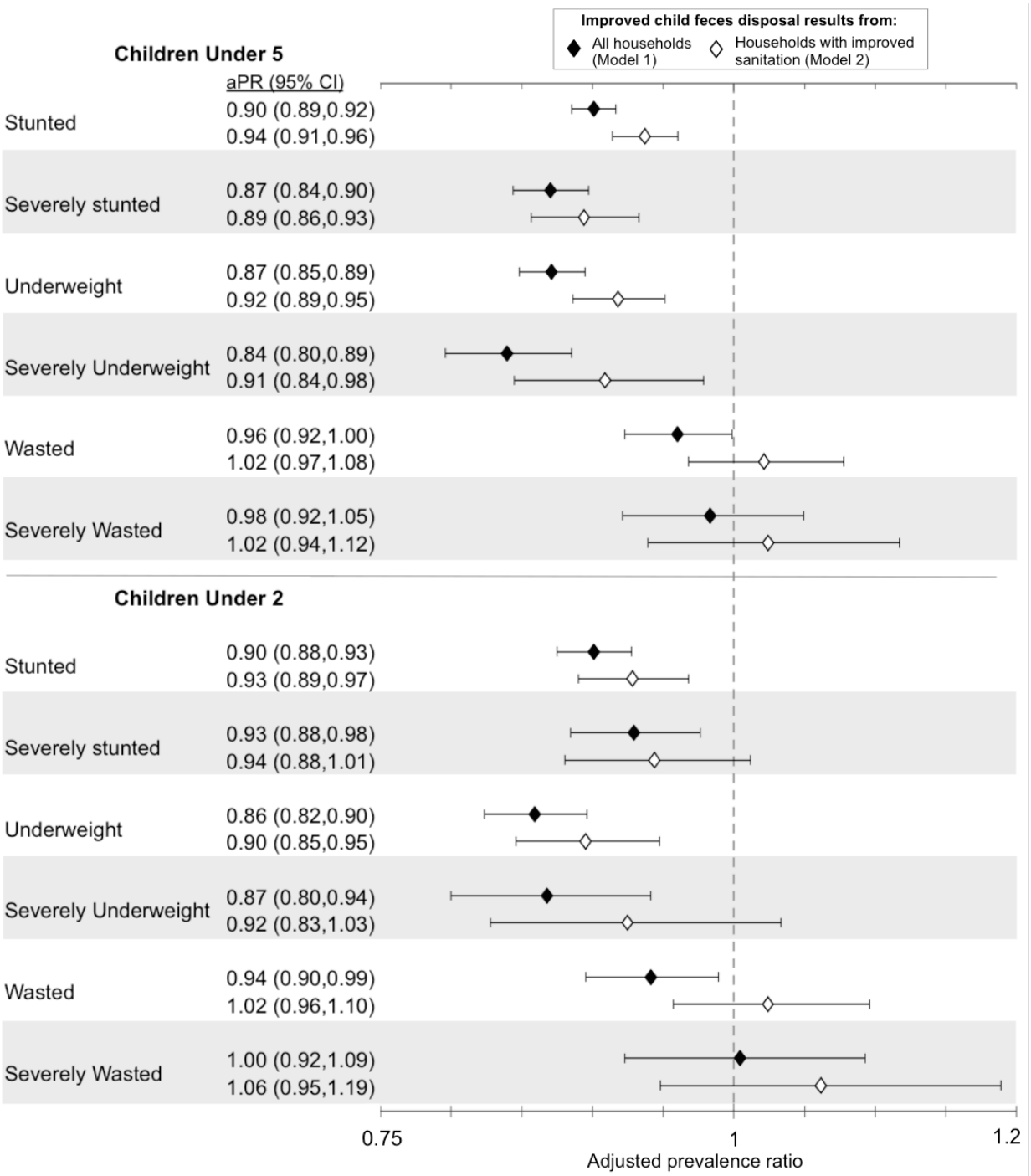


Figure 4.2. Adjusted prevalence ratios (aPRs) and 95% confidence intervals for undernutrition outcomes for children under 5 years old (top) and children under 2 years old (bottom) due to improved child faeces disposal practices in all households (Model 1 characteristics; filled diamonds) and households that have access to improved sanitation (Model 2 characteristics; empty diamonds). Each model includes country fixed-effects and has been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether

the child has a health card. Reductions in undernutrition outcomes associated with improved child faeces disposal in all households (Model 1 characteristics) evaluate the maximum benefits of these practices, whereas reductions in undernutrition outcomes in households with access to improved sanitation (Model 2 characteristics) evaluate the incremental benefits of child faeces management after improved toilet installation.

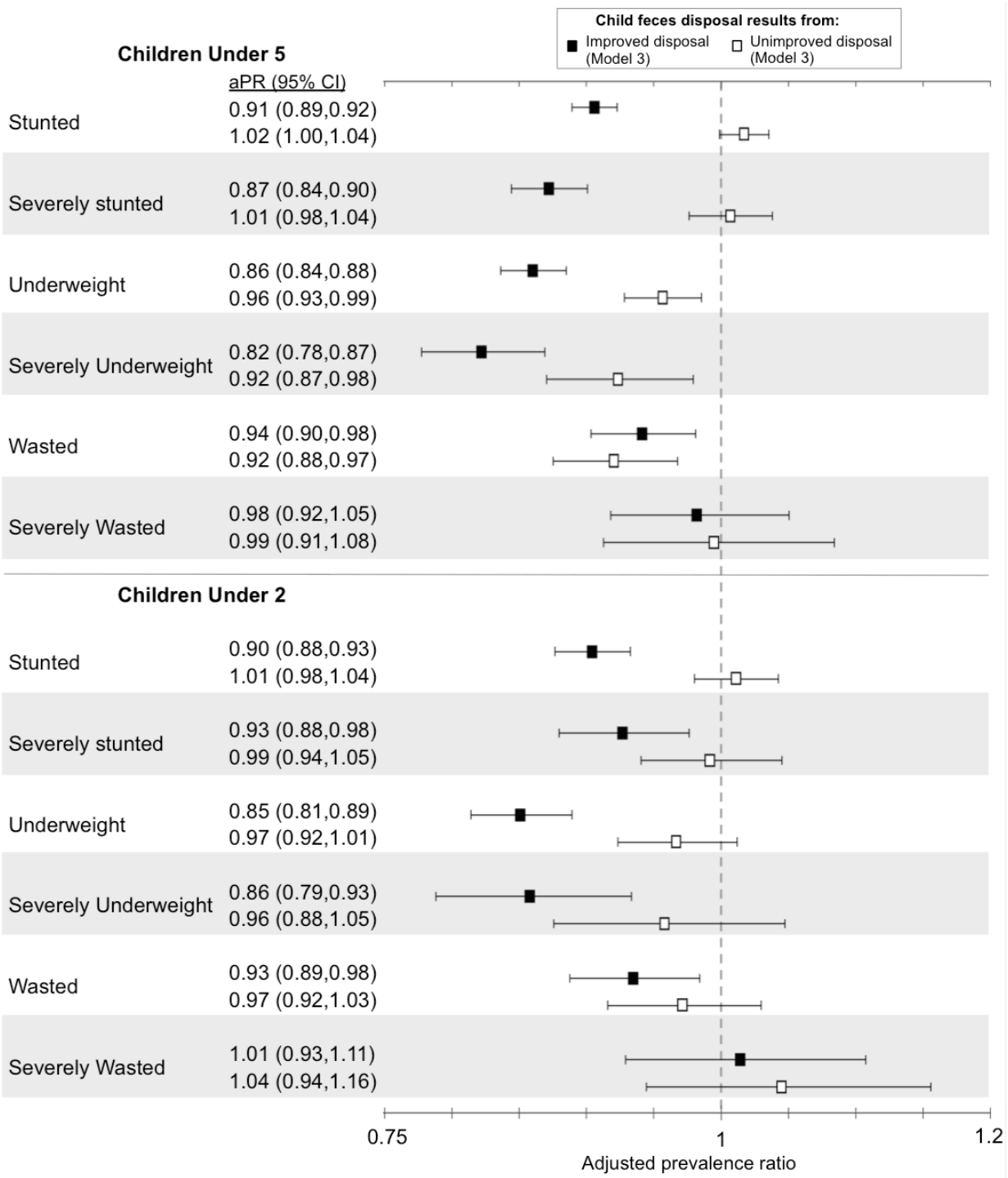


Figure 4.3. Adjusted prevalence ratios (aPRs) and 95% confidence intervals for undernutrition outcomes for children under 5 years old (top) and children under 2 years old (bottom) due to improved child faeces disposal practices (filled squares) and unimproved child faeces disposal practices (disposal in an unimproved toilet; empty squares) using Model 3 characteristics. Each model includes country fixed-effects and has been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital

status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.

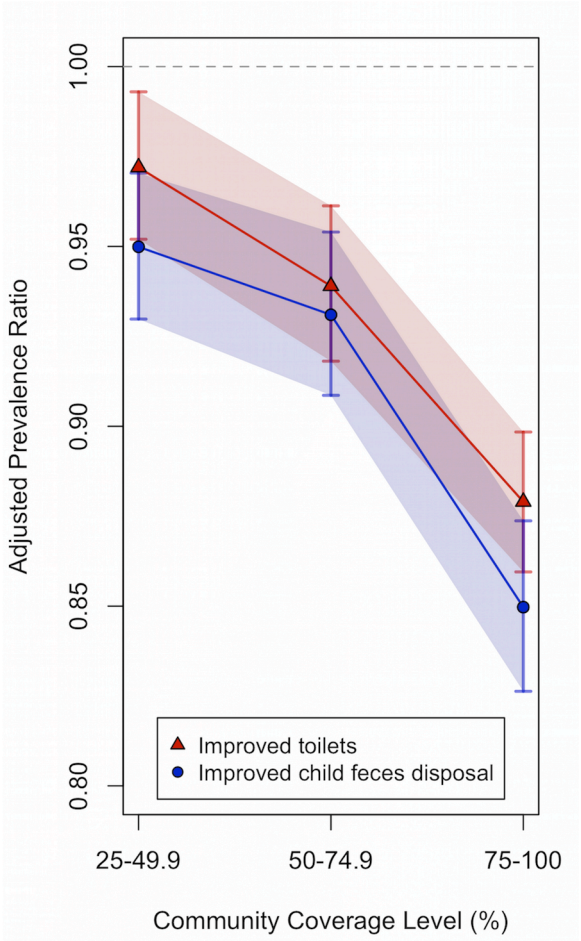


Figure 4.4. Adjusted prevalence ratios (aPRs) and 95% confidence intervals for stunting for children under 5 years old for community-coverage levels of improved toilets among households with children (red triangles; Model 4 characteristics) and improved child faeces disposal practices (blue circles; Model 7 characteristics). The model includes country fixed-effects and has been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.

4.7 References

1. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 2013: 382: 427–451
2. Olofin I, McDonald CM, Ezzati M, Flaxman S, Black RE, Fawzi WW, et al. Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: a pooled analysis of ten prospective studies. *PloS ONE* 2013: 8: e64636.
3. Caulfield LE, de Onis M, Blössner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am J Clin Nutr* 2004: 80: 193–198.
4. Guerrant RL, DeBoer MD, Moore SR, Scharf RJ, Lima AAM. The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease. *Nat Rev Gastroenterol Hepatol* 2012: 10: 220–229.
5. Pickering AJ, Djebbari H, Lopez C, Coulibaly M, Alzua ML. Effect of a community-led sanitation intervention on child diarrhoea and child growth in rural Mali: a cluster-randomised controlled trial. *Lancet Glob Health* 2015: 3: e701–e711.
6. Esrey SA. Water, waste, and well-being: a multicountry study. *Am J Epidemiol* 1996: 143: 608–623.
7. Checkley W, Gilman RH, Black RE, Epstein LD, Cabrera L, Sterling CR, et al. Effect of water and sanitation on childhood health in a poor Peruvian peri-urban community. *Lancet* 2004: 363: 112–118.
8. Lin A, Arnold BF, Afreen S, Goto R, Huda TMN, Haque R, et al. Household Environmental Conditions Are Associated with Enteropathy and Impaired Growth in Rural Bangladesh. *Am J Trop Med Hyg* 2013: 89: 130–137.
9. Fuller JA, Villamor E, Cevallos W, Trostle J, Eisenberg JN. I get height with a little help from my friends: herd protection from sanitation on child growth in rural Ecuador. *Int J Epidemiol* 2016: 45: 460-9.
10. Yeager BA, Huttly SR, Bartolini R, Rojas M, Lanata CF. Defecation practices of young children in a Peruvian shanty town. *Soc Sci Med* 1999: 49: 531–541.
11. Sultana R, Mondal UK, Rimi NA, Unicomb L, Winch PJ, Nahar N, et al. An improved tool for household faeces management in rural Bangladeshi communities. *Trop Med Int Health* 2013: 18:854–860.
12. Gil A, Lanata C, Kleinau E, Penny M. Strategic Report 11 Children's Feces Disposal Practices in Developing Countries and Interventions to Prevent Diarrheal Diseases. US Agency Int Dev Wash DC. 2004.
13. Baltazar JC, Solon FS. Disposal of faeces of children under two years old and diarrhoea incidence: a case-control study. *Int J Epidemiol* 1989: 18: S16–S19.
14. Bain R, Luyendijk R. Are burial or disposal with garbage safe forms of child faeces disposal? An expert consultation. *Waterlines* 2015: 34: 241–254.
15. Water and Sanitation Program/UNICEF. Management of Child Feces: Current Disposal Practices. 2015. Available: https://www.wsp.org/sites/wsp.org/files/publications/WSP-CFD-Summary-Brief.pdf [accessed 15 May 2016].
16. Majorin F, Freeman MC, Barnard S, Routray P, Boisson S, Clasen T. Child Feces Disposal Practices in Rural Orissa: A Cross Sectional Study. Goldhaber-Fiebert JD, editor. *PLoS ONE* 2014: 9: e89551.

17. Brown J, Cairncross S, Ensink JHJ. Water, sanitation, hygiene and enteric infections in children. *Arch Dis Child* 2013: 98: 629–634.
18. Roy E, Hasan KZ, Haque R, Haque AF, Siddique AK, Sack RB. Patterns and risk factors for helminthiasis in rural children aged under 2 in Bangladesh. *South Afr J Child Health* 2011: 5: 78–84.
19. George CM, Oldja L, Biswas S, Perin J, Sack RB, Ahmed S, et al. Unsafe Child Feces Disposal is Associated with Environmental Enteropathy and Impaired Growth. *J Pediatr* 2016: 176: 43-49.
20. Black RE, Allen LH, Bhutta ZA, Caulfield LE, De Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008: 371: 243–260.
21. Corsi DJ, Neuman M, Finlay JE, Subramanian SV. Demographic and health surveys: a profile. *Int J Epidemiol* 2012: 41: 1602–1613.
22. De Onis M. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. World Health Organization. 2006.
23. WHO/UNICEF. Progress on Sanitation and Drinking Water – 2015 Update and MDG Assessment. Geneva: World Health Organization. 2015.
24. Fink G, Günther I, Hill K. The effect of water and sanitation on child health: evidence from the demographic and health surveys 1986–2007. *Int J Epidemiol* 2011: 40: 1196–1204..
25. Arimond M, Ruel MT. Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health surveys. *J Nutr* 2004: 134: 2579–2585.
26. Smits J, Steendijk R. The international wealth index (IWI). *Soc Indic Res* 2015: 122: 65–85.
27. Checkley W, Buckley G, Gilman RH, Assis AM, Guerrant RL, Morris SS, et al. Multi-country analysis of the effects of diarrhoea on childhood stunting. *Int J Epidemiol* 2008: 37: 816–830.
28. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 2006: 367: 1521–1532.
29. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet* 2009: 374: 1032–1035.
30. Korpe PS, Petri WA. Environmental enteropathy: critical implications of a poorly understood condition. *Trends Mol Med* 2012: 18:328–336.
31. Lunn PG. The impact of infection and nutrition on gut function and growth in childhood. *Proc Nutr Soc* 2000: 59:147–154.
32. Mbuya MN, Humphrey JH. Preventing environmental enteric dysfunction through improved water, sanitation and hygiene: an opportunity for stunting reduction in developing countries. *Matern Child Nutr* 2016: 12: 106–120.
33. Hunter PR, Prüss-Ustün A. Have We Substantially Underestimated the Impact of Improved Sanitation Coverage on Child Health? A Generalized Additive Model Panel Analysis of Global Data on Child Mortality and Malnutrition. *PloS One* 2016: 11:e0164571.
34. Buttenheim AM. The sanitation environment in urban slums: implications for child health. *Popul Environ* 2008: 30:26–47.
35. Curtis V, Cousens S, Mertens T, Traore E, Kanki B, Diallo I. Structured observations of hygiene behaviours in Burkina Faso: validity, variability, and utility. *Bull World Health Organ* 1993: 71: 23.

36. Manun'Ebo M, Cousens S, Haggerty P, Kalengaie M, Ashworth A, Kirkwood B. Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. *Trop Med Int Health* 1997: 2:1015–1021.
37. Jinadu MK, Adegbenro CA, Esmai AO, Ojo AA, Oyeleye BA. Health promotion intervention for hygienic disposal of children's faeces in a rural area of Nigeria. *Health Educ J* 2007: 66:222–228.
38. Stanton BF, Clemens JD. An educational intervention for altering water-sanitation behaviors to reduce childhood diarrhea in urban Bangladesh II. A randomized trial to assess the impact of the intervention on hygienic behaviors and rates of diarrhea. *Am J Epidemiol* 1987: 125: 292–301.
39. Curtis V, Kanki B, Cousens S, Diallo I, Kpozehouen A, Sangare M, et al. Evidence of behaviour change following a hygiene promotion programme in Burkina Faso. *Bull World Health Organ* 2001: 79: 518–527.
40. Yeager BA, Huttly SR, Diaz J, Bartolini R, Marin M, Lanata CF. An intervention for the promotion of hygienic feces disposal behaviors in a shanty town of Lima, Peru. *Health Educ Res* 2002: 49: 531–541.
41. Miller-Petrie MK, Voigt L, McLennan L, Cairncross S, Jenkins MW. Infant and Young Child Feces Management and Enabling Products for Their Hygienic Collection, Transport, and Disposal in Cambodia. *Am J Trop Med Hyg* 2015: 94:456-465.

CHAPTER 5: SOIL INGESTION IS ASSOCIATED WITH CHILD DIARRHEA IN AN URBAN SLUM OF NAIROBI, KENYA[§]

5.1 Abstract

Diarrhea is a leading cause of mortality in children under five. We conducted a cross-sectional study of 54 children aged 3 months to 5 years old in Kibera, an urban slum in Nairobi, Kenya, to assess the relationship between caregiver-reported soil ingestion and child diarrhea. Diarrhea was significantly associated with soil ingestion (adjusted odds ratio 9.9, 95% confidence interval 2.1-47.5). Soil samples from locations near each household were also collected and analyzed for *Escherichia coli* and a human-associated *Bacteroides* fecal marker (HF183). *E. coli* was detected in 100% of soil samples (mean 5.5 log colony forming units [CFU] *E. coli* per gram of dry soil) and the *Bacteroides* fecal marker HF183 was detected in 93% of soil samples. These findings suggest that soil ingestion may be an important transmission pathway for diarrheal disease in urban slum settings.

5.2 Introduction

Diarrheal disease caused by fecal pathogens is the second leading cause of mortality in children under five, responsible for approximately 760,000 deaths annually.[1] Fecal pathogens are primarily transmitted through the fecal-oral pathway with commonly reported exposure points of water, fields/floor, hands, food, and flies.[2] However, soil ingestion due to exploratory mouthing

[§] This chapter has been published: Bauza, V., Ocharo, R. M., Nguyen, T. H., & Guest, J. S. (2017). Soil Ingestion Is Associated with Child Diarrhea in an Urban Slum of Nairobi, Kenya. *The American journal of tropical medicine and hygiene*, *96*(3), 569-575.

behavior of young children is a potentially important exposure pathway that has not been well studied or characterized.

Geophagy is the intentional ingestion of soil, and typically involves specific types of soil that are selected, prepared, and then eaten.[3] This behavior is practiced by both children and adults and has been found to be associated with micronutrient deficiency, anemia, enteric distress, soil-transmitted helminth infections, and hunger.[3–8] Nonetheless, the direction of the causal relationship for many of these associations and whether geophagy serves as a risky or protective behavior is still debated.[6,7] Beyond the learned behaviors of geophagy, soil ingestion is also an involuntary behavior as part of mouthing and exploration in young children. There are few studies related to exploratory soil ingestion by young children in low-income countries, but recent evidence[9,10] suggests that it may be an important exposure point for fecal contamination leading to child illness.

A recent study in rural Zimbabwe found that the majority of household soil samples were contaminated with the fecal indicator bacteria *Escherichia coli* and 13% of infants included in the study were observed to actively ingest this contaminated soil during a 6-hour structured observation period.[9] Another study in peri-urban Tanzania found *E. coli* pathotype genes, a human-specific *Bacteroidales* gene, and enteric viruses present in a subset of soil samples taken from household locations, demonstrating that household soils may be contaminated with fecal pathogens.[11] Soil ingestion by young children has also been linked to negative health outcomes and was found to be associated with increased risk of diarrhea in rural Kenya,[12] and with environmental enteropathy and stunting in rural Bangladesh.[10] However, these studies linking

soil ingestion with negative health outcomes for young children were conducted in rural areas and do not provide a comprehensive understanding of the consequences of soil ingestion. Little is known about the prevalence and effects of exploratory soil ingestion in young children in urban areas. Studying low-income urban areas is important because the higher population density leads to increased foot traffic on soil close to households, the types and density of sanitation facilities vary relative to rural areas, and the drainage infrastructure is often poor in urban slums. These factors may impact the quantity and frequency of fecal pathogens released to the environment, affecting soil contamination. For example, in the previous study in rural Kenya that demonstrated a link between soil ingestion and diarrhea, 40% of households included in the study did not have access to any latrine,[12] whereas open defecation by adults is rare in urban areas (practiced by 3% of urban households in Kenya in 2015).[13]

The objective of this study was to assess the relationship between caregiver reported soil ingestion events and diarrhea episodes in children under five residing in Kibera, an urban slum of Nairobi, Kenya. In order to better understand factors influencing this relationship, fecal contamination levels of soil samples collected near each household were also measured by enumerating *E. coli* via membrane filtration and quantifying a human-associated *Bacteroides* fecal marker using quantitative polymerase chain reaction (qPCR). This is the first time that the link between soil ingestion and diarrhea has been studied in urban areas and in households with primarily non-earth floors, as well as the first time a human-associated fecal marker has been measured in soil in this setting to determine whether human fecal contamination may be a significant contamination source of soil in urban slum areas.

5.3 Methods

Study site and sampling frame

This study was conducted in June 2015 in the Makina, Sarangombe, and Lindi wards of Kibera, the largest urban slum in Nairobi, Kenya. Purposeful sampling was used to select slum compounds (clusters of households with shared common areas and often shared toilets and water sources) that were in different wards and different areas within a ward to increase the variation in sanitation, drainage, and solid waste infrastructure for compounds included in this study. Within each compound, households with children under five were then randomly selected for study inclusion, approached for informed consent, and household interviews were conducted with the primary caregiver to obtain information about household demographics, behaviors, and child health information. A total of 54 children (aged 3 months to <5 years) from 40 households were included in this study, from 16 different compounds. The results presented here are part of a larger study of infrastructure and practices related to water, sanitation, and hygiene in Kibera.

Ethical approval

Informed consent was obtained from the household's primary caregiver prior to enrollment. The study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign and the National Commission for Science, Technology and Innovation in Kenya.

Household interviews

A questionnaire on the household's socio-demographic characteristics, diarrhea illness occurring in the past week for all children in the household, and infrastructure and behaviors related to water, sanitation, and hygiene was administered to each household's primary caregiver by a

Kenyan interviewer in Kiswahili or Dholuo (depending on the respondent's primary language). Diarrhea was defined as having three or more bowel movements within 24 hours. For each child, caregivers were also asked if they had observed that child putting soil, mud, clay, or sand into his or her mouth in the past 7 days. This question was asked because caregiver reported soil ingestion/geophagy events have been previously found to show good agreement with soil ingestion events observed during structured observation in Bangladesh.[10]

Soil sampling

Soil samples were collected from outdoor locations near each household entrance, for a total of 28 samples. Since a number of households had shared outdoor common space that the household entrance opened into, this provided soil samples from locations near 34 households. Soil was sampled from outside each household because only one household included in the study had an earth floor and the remaining households had a hard floor (consisting of either vinyl, concrete, wood, or tile). To be consistent among households, soil was not collected for households surrounded by concrete walkways (N=6) because there was no soil near the household entrance with which a child would be likely to come in frequent contact with and consume. Soil samples were collected using sterile polystyrene sampling spoons (Nasco, Fort Atkinson, WI) such that approximately 10 grams of soil were collected from the surface of soil (from an area approximately 10 cm by 10 cm) and placed in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) for transport to the laboratory in a cooler with ice packs. At the time of sampling, ambient temperature ranged from 21^{o}C to 29^{o}C and relative humidity ranged from 41% to 76%. All samples were taken from areas that were either fully or partially shaded from sunlight.

E. coli enumeration

Samples were processed within 8 hours of sample collection. Each soil sample was homogenized inside of a sterile bag by hand. 2 grams of soil were then hand shaken for 2 minutes in 20 mL of phosphate buffered saline (PBS), and allowed to settle for 30 seconds, following the methods recommended by Boehm et al.[14] to recover *E. coli*. The supernatant was then poured into a sterile container, diluted, and filtered through 0.45 µm pore size (47 mm diameter) mixed cellulose esters filters (Pall Corporation, Port Washington, NY). *E. coli* was enumerated using m-ColiBlue24 Broth media (Hach, Loveland, CO) following the manufacturer's protocol approved by the U.S. Environmental Protection Agency (EPA). Plates were incubated at 35^{o}C for 24 hours. Filtration blanks were processed daily. Additionally, 5 grams of soil were dried in an oven at 105^{o}C for 24 hours to determine moisture content.

Molecular analysis for human-specific *Bacteroides* detection

DNA was extracted from approximately 0.25 grams of soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) following manufacturer's guidelines. The DNA extract was stored at -20^{o}C for up to 3 weeks, transported back to the University of Illinois in a cooler with ice packs, and stored at -80^{o}C until further analysis.

Nucleic acid extracts were analyzed via qPCR for the human-associated *Bacteroides* fecal marker HF183, which has been previously validated in Kenya.[15] 15 µL reactions were used for the HF183 assay, containing 1X final concentration of SYBR Green I dye master mix (Applied Biosystems, Waltham, MA) and 250 nM of forward and reverse primers. Previously published primers that have been verified in Kenya were used.[16] Samples were amplified in a 384-well

plate on an Applied Biosystems 7900HT Fast Real-Time PCR System with the following thermocycle conditions: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 45 sec at 53°C, and 1 min at 60°C. A dissociation curve analysis was performed with conditions set to 15 sec at 95°C, 20 sec at 60°C, and 15 sec at 95°C to determine the melting temperature of amplified sequences in each sample. Seven 10-fold serial dilutions (3×10^0 to 3×10^6 genes copies/μL) of the target synthesized DNA sequence were used to calculate amplification efficiency and gene copies per μL DNA extract from quantification cycle threshold (C_q) values. Standard curve dilutions were run in triplicate, environmental samples were run in duplicate, and three no-template controls were run on each plate. The target HF183 sequence was considered detected if amplification was observed in the sample well and the melting temperature was between 73°C and 76°C. The lower limit of quantification (LLOQ) was defined as the lowest concentration at which more than 50% of standard curve replicates were amplified.[17] Positive samples were considered within the range of quantification (ROQ) if the C_q was above the LLOQ (3 gene copies/μL, C_q<32). The remaining samples that detected the target HF183 sequence were classified as detected, but not quantified (DNQ). The average LLOQ was 1,463 gene copies per gram of dry soil. Assuming a theoretical minimum detection of 3 copies per PCR reaction,[18] the average limit of detection (LOD) is 732 gene copies per gram of dry soil. The LOD was assigned to samples classified as DNQ and half the LOD was assigned to samples with the target not detected (ND) for quantitative analysis.

PCR inhibition was assessed by examining the linearity of response in the C_q values across two fivefold dilutions of DNA extract, following the method in Cao et al.[19] For samples classified as quantifiable (C_q < 32), fivefold dilutions were completed using each sample's DNA extract. For

samples that were classified as DNQ or ND, a subset of these samples were evaluated for inhibition by spiking 3×10^3 copies/µL into the DNA extract prior to completing fivefold dilutions to measure the linearity of response. Samples were considered inhibited if the ΔC_q between fivefold dilutions was less than 1.3, which is one cycle less than expected amplification assuming 100% efficiency, as recommended by Cao et al.[19]

Statistical analysis

Stata version 13.1 was used for all data analysis with the primary goal of determining if child soil ingestion was associated child diarrhea in the past 7 days. Chi-squared, Fisher's exact tests, and penalized maximum likelihood logistic regression (firthlogit command in Stata) were used to analyze associations between child diarrhea and child soil ingestion. Penalized maximum likelihood logistic regression was also used to control for potential confounding variables and obtain adjusted odds ratios (ORs). Penalized maximum likelihood logistic regression was used because this method addresses the problem of small sample size/sparse-data bias that can overestimate odds ratios for small sample sizes when using ordinary multivariable logistic regression.[20,21]

The following potential confounding variables were assessed for inclusion in the analysis: child's age, asset ownership index (to represent household income), number of household members, mother's educational attainment, drinking water contamination at time of visit, and whether the feces of all children under five in the household are disposed of in a toilet or latrine. The drinking water contamination variable is a binary variable for whether any *E. coli* contamination was detected in a 100 mL sample of drinking water collected at the time of the household visit.

The asset ownership index variable is an integer variable indicating the number of the following assets the household owns (or uses, in the case of electricity): bicycle, motorcycle, radio, television, computer, bed, wardrobe, electricity, livestock. Variables for the multiple variable model were selected if their association with child diarrhea had a p-value less than 0.2 in the binary or multiple variable model. The assets index and number of household members variables met these criteria and were included in adjusted models.

Two-sample t-tests were used to test for statistical associations using the *E. coli* and HF183 measurements. Soil *E. coli* colony forming units (CFU) data and HF183 gene copy were log transformed before analysis to normalize their log normal distribution. For samples with *E. coli* counts that were too numerous to count (>500 CFU per filter), 550 CFU per filter volume was used.

Additionally, in order to enable a comparison with a previous study in rural Kenya,[12] Pearson's correlation coefficient was calculated to test the association between caregiver-reported child diarrhea and caregiver-reported child soil ingestion.

5.4 Results

Household characteristics

The average household size was 5.25 (standard deviation [SD] 1.9, range 3-10) people, with an average of 1.4 (SD 0.5, range 1-3) children under five. The majority of female heads of the household (55%, N=22) had completed primary education or fewer years of schooling. The average reported monthly household income of respondents was $105 USD (SD 65, range 20-

300). 17 households (42.5%) used a facility with a pour flush toilet to sewer, 20 households (50%) used a pour flush pit latrine or a pit latrine with slab, and 3 households (7.5%) used a pit latrine without slab. 95% of these toilet facilities were shared with other households.

Child soil ingestion and diarrhea

Caregivers reported observing their child put soil into their mouth in the past 7 days for 44.4% of children (N=24) included in the study. Soil ingestion was reported most frequently for children in the 6-24 month age group, and the prevalence of reported soil ingestion decreased with increased age. It was reported least frequently for children aged 3-5, but was still reported for one third of children in this age group. Diarrhea was reported for 24.1% of all children (N=13), with the highest levels among children aged 6-24 months and prevalence decreasing with age (Table 5.1).

Children who had been observed to ingest soil in past 7 days were significantly more likely to have had diarrhea in the past week compared to children who were not observed to ingest soil (χ^2=11.2, p=0.001). Soil ingestion and diarrhea were also significantly associated with each other (Pearson's r=0.46, p=0.0005). Penalized maximum likelihood logistic regression results (Table 5.2) revealed the odds of diarrhea were more than 9 times higher for children who were observed to ingest soil in the past week compared to those who were not (adjusted OR = 9.9, 95% confidence interval [CI]: 2.1 – 47.5). This association was higher among children aged 6 months to <36 months, who were more likely to consume soil than other children (adjusted OR = 12.9, 95% CI: 1.9 – 88.5).

Soil contamination

E. coli was detected in every soil sample, and samples had a mean of 5.5 log CFU *E. coli* (SD 0.35) per gram of dry soil. The human-associated *Bacteroides* fecal marker HF183 was detected in 93% of samples (N=26). Overall, 36% of samples were quantifiable (N=10), 57% of samples were DNQ (N=16), and 7% of samples were ND (N=2) for the HF183 target. Among samples within the ROQ, the HF183 gene had a mean of 4.2 log copies (SD 0.5) per gram of dry soil. There was no correlation between *E. coli* levels and HF183 levels found in soil samples (Pearson's r=0.06, p=0.79).

Households with a child who was observed to consume soil were more likely to have lower levels of HF183 copies in soil samples (Table 5.3, mean of 3.6 log copies for households without a child observed to put soil in their mouth vs. 3.1 log copies for households with a child observed to put soil in their mouth, t=2.3, p=0.03). However, there was no statistically significant difference in *E. coli* counts (mean of 5.6 log CFU *E. coli* for households without a child observed to put soil in their mouth vs. 5.5 log CFU *E. coli* for households with a child observed to put soil in their mouth, t=0.71, p=0.49).

Among households in which at least one child was reported to ingest soil, there was no statistically significant difference in the average *E. coli* count in soil samples from households with at least one child reported to have had diarrhea in the past week (mean of 5.6 log CFU *E. coli* vs. 5.4 log CFU *E. coli* for households with children without diarrhea, t=-1.04, p=0.35) or in HF183 copies in soil samples from these two groups of households (mean of 3.2 log copies vs. 3.0 log for households with children without diarrhea, t=-0.56, p=0.59).

Similarly, among all households, there was no statistically significant difference in the average *E. coli* count in soil samples from households with children reported to have had diarrhea in the past week (Table 5.3, mean of 5.7 log CFU *E. coli* vs. 5.5 log CFU *E. coli* for households with children without diarrhea, t=-1.69, p=0.13) or in the average HF183 genes copies in soil samples (mean of 3.1 log copies for households with children reported to have had diarrhea in the past week vs. 3.4 log copies for households with children without diarrhea, t=1.07, p=0.30).

Quality assurance and control

All filtration blanks, extraction blanks, and no template controls were negative. Linearity (R^2=0.99) and efficiency (e=103%) of the HF183 qPCR assay were within acceptable ranges. Minor PCR inhibition was detected between undiluted and 1:5 dilutions of DNA extract, but, with the exception of two samples, no inhibition was detected between 1:5 and 1:25 dilutions. As a result, C_q results from the 1:5 dilutions were used to quantify the number of gene copies in each sample.

5.5 Discussion

This study demonstrated an association between soil ingestion and child diarrhea in an urban slum environment. This adds to the growing body of knowledge that existing water, sanitation, and hygiene (WASH) interventions that target improving water access, toilet/latrine access, and handwashing may not eliminate children's exposure to fecal contamination in the domestic environment.[9,10,22,23] Reported soil ingestion was the highest among children aged 6 to 24 months, which is consistent with a previous studies evaluating child geophagy[10] and child

mouthing behavior.[24] Additionally, no soil ingestion was reported for children younger than 6 months, which is likely due to children being relatively inactive at this age.

Our results showed a positive association with soil ingestion and child diarrhea in a densely populated, urban slum area of Kenya, which is consistent with a previous study in rural Kenya[12] that also assessed this relationship. While our study found a higher association of soil ingestion with diarrhea (Pearson's correlation of r=0.46 between soil ingestion and diarrhea) than the previous study in rural Kenya (significant Pearson's correlation of r=0.306),[12] this may indicate that soil ingestion is a higher risk activity in urban slum areas, but may also be a result of the relatively small sample sizes in both studies. Notably, however, our study demonstrated a link between soil ingestion and diarrhea in households without earth floors (only one household included in our study had an earth floor in the home). As there is soil in the immediate environment surrounding many of the houses in this slum and children are not constantly restricted to staying inside the houses, child exposure to soil is likely even in households without earth floors. To our knowledge, this is the first study demonstrating this link in a study population with primarily non-earth household flooring. This finding indicates that soil ingestion may be an important exposure route for children regardless of household floor material, and upgrading earth floors to concrete or other non-earth materials may not eliminate soil as an exposure route for fecal contamination.

Soil fecal contamination measured in this study was also higher than past studies. Soil sampled in this study had a mean of 5.5 log CFU *E. coli* per gram of dry soil, which is higher than the reported means of 4.4 log CFU *E. coli* per gram of wet soil in an urban slum in Uganda,[23] 3.85

log CFU *E. coli* per gram of soil in rural Bangladesh,[10] 2.1 log CFU *E. coli* per gram of dry soil in peri-urban Tanzania,[11] and 1.84 log CFU *E. coli* per gram of soil from a yard laundry area in rural Zimbabwe during dry season.[9] However, the study in an urban slum in Uganda was conducted during dry season and used an augur to collect soil from the top 15 cm of soil, which is different from this study in which soil was collected only from the surface where children would come into contact with it and soil may have higher levels of contamination. Samples in our study were also taken during June, during the wet season when there may be higher fecal contamination exposure than in the dry season. High soil moisture and flooding have been linked with greater survival of *E. coli* in soils,[25] which may result in an increase of fecal contamination of soil during the wet season compared to the dry season. Although additional studies would be beneficial for providing more conclusive comparisons between the relative amount of soil contamination in these areas, our results indicate that fecal contamination of soil may be higher in urban slums than rural and peri-urban areas. This finding demonstrates the importance of using fecal contamination exposure estimates specific to urban slums when conducting risk assessments in these settings, as using values measured in other areas will likely underestimate the level of fecal contamination exposure. Furthermore, it is critical to include soil as an exposure point in fecal pathogen exposure risk assessment, and the high variability among fecal contamination levels measured in soil from different study locations warrants site-specific soil measures be conducted as part of exposure assessments.

The human-associated *Bacteroides* fecal marker HF183 was detected in 93% of soil samples (N=26), indicating that human fecal contamination of the soil sampled was likely. The specific human-associated *Bacteroides* marker chosen for this study (HF183 SYBR) has been previously

validated in Kenya with a sensitivity of 65% and specificity of 100%.[15] Within the study area, there are several potential sources that could cause soil to become contaminated with human feces. Leaking pit latrines, unhygienic disposal of feces from young children (including large garbage piles that contained used diapers), and open drainage ditches are all potential sources of human fecal contamination that we observed within the study area. Flooding in households and shared outdoor spaces where soil was sampled was common, with 60% of households reporting flooding within the past month (N=24). Rainfall and flooding may cause feces from these potential sources to disperse and spread human feces around the environment, contaminating nearby soil. More research is needed to determine the relative contribution of each potential human contamination source (leaking pit latrines, unhygienic child feces disposal, open drainage ditches) to the contamination of soil near households. However, these infrastructure and behavior characteristics may also explain why we measured higher levels of fecal contamination in this urban slum setting compared to fecal contamination levels previously measured in rural and peri-urban areas.

Although we did not use direct observation to measure the quantity of soil consumed by children as part of this study, it is reasonable to leverage published estimates of soil quantity consumed by young children to estimate fecal contamination exposure by soil ingestion in our study population. In rural Zimbabwe, it was estimated that children who intentionally consumed soil consumed 1.25 grams of soil per episode, with a mean of 11.3 soil-mouth episodes during a six-hour observation period.[9] This would be equivalent to children ingesting roughly 395,000 *E. coli* per episode in our study site. Although only certain strains of *E. coli* are capable of producing illness in humans, the detection of human-specific *Bacteroides* genetic markers have been shown

to be predictive of the presence of pathogenic *E. coli* in surface waters in Japan.[26] As the human-specific *Bacteroides* HF183 genetic marker was detected in 93% of soil samples collected in this study, it is likely that pathogenic *E. coli* are present in the soil, but it is also possible that the relationship seen in surface waters between human-specific *Bacteroides* and pathogenic *E. coli* may not be representative of the relationship in soil samples collected from our study site. The presence of pathogenic *E. coli* can be estimated in the samples collected by assuming that 8% of *E. coli* detected were pathogenic, which has been previously recommended as a low-cost method of quantifying risk from water supplies in resource-limited settings.[27] This would correspond to children ingesting roughly 31,600 pathogenic *E. coli* per soil mouthing episode in our study site. While the specific estimate for the quantity of pathogenic *E. coli* consumed should be interpreted with caution as many assumptions were made in the calculation and pathogenic genes were not measured directly in this study, this rough assessment illustrates that soil is likely a significant route for fecal contamination exposure in children.

In this study, we also found that households with lower levels of human fecal contamination in soil samples (quantified by copies of the *Bacteroides* HF183 gene) were more likely to have a child who was observed to consume soil. This may indicate that caregivers living in unclean environments with greater human fecal contamination are more likely to stop their children from putting soil in their mouth. Similarly, it may indicate that caregivers who are aware that their children put soil in their mouth are more likely to keep the soil near the household cleaner and dispose of waste somewhere else. However, in-depth interviews or focus groups would be necessary to confirm this finding.

Although this study supports the general hypothesis that soil ingestion may be an important transmission pathway for diarrheal disease in urban slum settings, this study also has notable limitations. Since this is a cross-sectional study, a causal relationship between soil ingestion and diarrhea could not be determined. This study also had a small sample size, which limited the statistical power. Some potential confounding variables were included in the analysis by using penalized maximum likelihood logistic regression, but there may be other confounding factors that were not included in the model that could influence the results and increasing the sample size in future studies could allow more confounding variables to be controlled for. Additionally, although soil was found to contain high levels of fecal contamination which may link soil ingestion with diarrhea, children who put soil in their mouth may also be more likely to put other contaminated objects into their mouth. It is possible that this mouthing/exploratory behavior may lead children to mouth or ingest other contaminated objects that cause diarrhea. In that case, reported soil ingestion could potentially be a proxy of high-risk mouthing behaviors. Furthermore, this study relied on information reported by caregivers, which could lead to reporting bias. Although a previous study showed good agreement between caregiver reported and directly observed soil ingestion by children,[10] using structured observation in addition to reported data could improve accuracy. If structured observation is not feasible, future studies should include questions related to the frequency, location, and quantity of soil observed by caregivers to be consumed by children for exposure estimates. Future studies also could improve upon this work by increasing the sample size to improve the generalizability of the results and provide greater statistical power to identify associations between soil contamination and soil properties, household behaviors, and infrastructure characteristics. Additionally, future studies could inform educational intervention design by including questions related to whether the

caregiver perceives soil ingestion by children as a risky behavior or not (and why the caregiver feels this way), as well as if the caregiver stops the child from ingesting soil when it is observed.

Despite recent evidence that soil ingestion may be an important exposure point for fecal pathogens, interventions aimed at reducing exposure from exploratory soil ingestion are limited. A randomized controlled trial in rural Zimbabwe, referred to as the SHINE trial, is currently being conducted to evaluate the use of hygienic play spaces that are mobile and placed over soil to reduce contact with fecal contamination in the environment.[28] The evaluation of additional interventions is needed, including interventions to create a barrier between fecal pathogens in the environment and children in low-income urban areas, as well as educational interventions to reduce risky child mouthing behavior with soil. A recent study in Bangladesh identified that caregivers rarely intervene when children are ingesting soil, stopping children from soil ingestion only 14% of the time during direct observation.[10] Young children require close supervision, and the presence or lack of supervision may be informed by a caregiver's child-rearing attitude, including whether limits set for children are permissive or restrictive.[29] However, it is not well understood if the low frequency at which parents have been observed to intervene with child soil consumption is due to the caregiver's child rearing attitudes, a lack of knowledge about risks associated with a child ingesting soil, or other factors. A better understanding of the factors that determine whether or not a caregiver intervenes during child soil ingestion would be useful for designing better interventions. Additionally, improvements in sanitation infrastructure, drainage infrastructure, child feces disposal practices, and solid waste management could also reduce soil contamination from human fecal sources.

This study provides further evidence that soil is an important pathway for fecal pathogen exposure for children in low-income environments, as well as evidence that soil is still an important exposure pathway for children living in households with non-earth flooring. The results also suggest that soil ingestion may be a more important exposure pathway for children in urban slums than in rural areas as the urban environment soil had higher levels of contamination and soil ingestion had a stronger association with diarrhea. However, as there is limited data available for each of these settings, it is also possible that other site-specific characteristics may influence soil contamination more than the general classification of rural or urban setting and site-specific soil measures are recommended for future exposure assessments. Further research is also needed to evaluate interventions that limit soil ingestion behavior in children in both rural and urban areas. Reducing soil ingestion by children should be included in holistic approaches to improve WASH that move beyond conventional water and sanitation access.

5.6 Acknowledgements

The authors thank Phantus Wambiya, Rodah Obondi, and Philip Omandid for their help conducting household interviews and fieldwork, Gabrielle Levato for her help with field sampling and lab work, Professor Vincent Madadi at the University of Nairobi for providing laboratory space, and Professor Juliet Iwelunmor at the University of Illinois for helpful discussions. We also thank the participating households who made this study possible.

5.7 Financial Support

Valerie Bauza was supported by the National Science Foundation Graduate Research Fellowship Program under grant no. DGE-1144245 while conducting this work, and travel and supplies were

supported by the Safe Global Water Institute (SGWI) and the Department of Civil and Environmental Engineering at the University of Illinois at Urbana-Champaign.

Table 5.1. Child outcome characteristics for soil ingestion and diarrhea in the past 7 days.

	Total	Reported Soil Ingestion (N [% of Age Group])	Reported Diarrhea (N [% of Age Group])		
			All	Soil Ingestion	No Soil Ingestion
All children included in study	54	24 (44.4%)	13 (24.1%)	11 (20.4%)	2 (3.7%)
Children 3-6 months	4	0 (0%)	0 (0%)	-	-
Children 6-24 months	24	14 (58.3%)	9 (37.5%)	8 (33.3%)	1 (4.2%)
Children 24-36 months	14	6 (42.9%)	3 (21.4%)	3 (21.4%)	0 (0%)
Children 3-5 years	12	4 (33.3%)	1 (8.3%)	0 (0%)	1 (8.3%)

Table 5.2. Association between child soil ingestion and diarrhea from penalized maximum likelihood logistic regression models.

Child diarrhea explanatory variable	Unadjusted			Adjusted		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Children aged 3 months to <5 years (N=54)						
Soil ingestion	9.7	2.1 – 44.1	0.003	9.9	2.1 – 47.5	0.004
Asset index				0.7	0.4 – 1.2	0.17
# of household members				1.4	0.9 – 2.1	0.13
Children aged 6 months to <36 months (N=38)						
Soil ingestion	14.1	2.2 – 92.2	0.006	12.9	1.9 – 88.5	0.009
Asset index				0.7	0.4 – 1.2	0.22
# of household members				1.4	0.84 – 2.3	0.19

Table 5.3. Mean and standard deviation for *E. coli* CFU and HF183 gene copies per dry gram of soil for the households and p-values from two-sample t-tests. Values reported as mean (SD).

	Child ingesting soil in household?			Child with diarrhea in household?		
	Yes (N=18)	No (N=16)	p-value	Yes (N=9)	No (N=25)	p-value
E. coli (log CFU/gram of dry soil)	5.5 (0.4)	5.6 (0.3)	0.49	5.7 (0.3)	5.5 (0.4)	0.13
HF183 gene (log copies/gram of dry soil)	3.1 (0.6)	3.6 (0.8)	0.03	3.1 (0.6)	3.4 (0.8)	0.30

5.8 References

1. World Health Organization, 2013. Diarrhoeal disease fact sheet. Available at: http://www.who.int/mediacentre/factsheets/fs330/en/. Accessed December 15, 2015.
2. Curtis V, Cairncross S, Yonli R, 2000. Review: Domestic hygiene and diarrhoea–pinpointing the problem. *Trop Med Int Health 5*: 26-30.
3. Kutalek R, Wewalka G, Gundacker C, Auer H, Wilson J, Haluza D, Huhulescu S, Hillier S, Sager M, Prinz A, 2010. Geophagy and potential health implications: geohelminths, microbes and heavy metals. *Trans R Soc Trop Med Hyg 104*: 787-795.
4. Young SL, Khalfan SS, Farag TH, Kavle JA, Ali SM, Hajji H, Rasmussen KM, Pelto GH, Tielsch JM, Stoltzfus RJ, 2010. Association of Pica with Anemia and Gastrointestinal Distress among Pregnant Women in Zanzibar, Tanzania. *Am J Trop Med Hyg 83*: 144-151.
5. Luoba AI, Wenzel Geissler P, Estambale B, Ouma JH, Alusala D, Ayah R, Mwaniki D, Magnussen P, Friis H, 2005. Earth-eating and reinfection with intestinal helminths among pregnant and lactating women in western Kenya. *Trop Med Int Health 10*: 220-227.
6. Young SL, Sherman PW, Lucks JB, Pelto GH, 2011. Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. *Q Rev Biol 86*: 97-120.
7. Miao D, Young SL, Golden CD, 2015. A meta-analysis of pica and micronutrient status: Pica And Micronutrient Meta-Analysis. *Am J Hum Biol 27*: 84-93.
8. Geissler PW, Mwaniki DL, Thiong'o F, Michaelsen KF, Friis H, 1998. Geophagy, iron status and anaemia among primary school children in Western Kenya. *Trop Med Int Health 3*: 529-534.
9. Ngure FM, Humphrey JH, Mbuya MNN, Majo F, Mutasa K, Govha M, Mazarura E, Chasekwa B, Prendergast AJ, Curtis V, Boor KJ, Stoltzfus RJ, 2013. Formative Research on Hygiene Behaviors and Geophagy among Infants and Young Children and Implications of Exposure to Fecal Bacteria. *Am J Trop Med Hyg 89*: 709-716.
10. George CM, Oldja L, Biswas S, Perin J, Lee GO, Kosek M, Sack RB, Ahmed S, Haque R, Parvin T, Azmi IJ, Bhuyian SI, Talukder KA, Mohammad S, Faruque AG, 2015. Geophagy is associated with environmental enteropathy and stunting in children in rural Bangladesh. *Am J Trop Med Hyg 92*: 1117-1124.
11. Pickering AJ, Julian TR, Marks SJ, Mattioli MC, Boehm AB, Schwab KJ, Davis J, 2012. Fecal Contamination and Diarrheal Pathogens on Surfaces and in Soils among Tanzanian Households with and without Improved Sanitation. *Environ Sci Technol 46*: 5736-5743.
12. Shivoga WA, Moturi WN, 2009. Geophagia as a risk factor for diarrhoea. *J Infect Dev Ctries 3*: 094-098.
13. WHO/UNICEF, 2015. Progress on Sanitation and Drinking Water – 2015 Update and MDG Assessment. Geneva and New York: World Health Organization and UNICEF.
14. Boehm AB, Griffith J, McGee C, Edge TA, Solo-Gabriele HM, Whitman R, Cao Y, Getrich M, Jay JA, Ferguson D, Goodwin KD, Lee CM, Madison M, Weisberg SB, 2009. Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *J Appl Microbiol 107*: 1740-1750.
15. Jenkins MW, Tiwari S, Lorente M, Gichaba CM, Wuertz S. Identifying human and livestock sources of fecal contamination in Kenya with host-specific Bacteroidales assays, 2009. *Water Res 43*: 4956-4966.

16. Seurinck S, Defoirdt T, Verstraete W, Siciliano SD, 2005. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environ Microbiol 7*: 249-259.
17. Layton BA, Cao Y, Ebentier DL, Hanley K, Ballesté E, Brandão J, Byappanahalli M, Converse R, Farnleitner AH, Gentry-Shields J, Gidley ML, Gourmelon M, Lee CS, Lee J, Lozach S, Madi T, Meijer WG, Noble R, Peed L, Reischer GH, Rodrigues R, Rose JB, Schriewer A, Sinigalliano C, Srinivasan S, Stewart J, Van De Werfhorst LC, Wang D, Whitman R, Wuertz S, Jay J, Holden PA, Boehm AB, Shanks O, Griffith JF, 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Res 47*: 6897-6908.
18. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem 55*: 611-622.
19. Cao Y, Griffith JF, Dorevitch S, Weisberg SB, 2012. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *J Appl Microbiol 113*: 66-75.
20. Heinze G, Puhr R, 2010. Bias-reduced and separation-proof conditional logistic regression with small or sparse data sets, *Stat Med 29*: 770-777.
21. Heinze G, Schemper M, 2002. A solution to the problem of separation in logistic regression. *Stat Med*. *21*: 2409-2419.
22. Zambrano LD, Levy K, Menezes NP, Freeman MC, 2014. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. *Trans R Soc Trop Med Hyg 108*: 313-325.
23. Katukiza AY, Ronteltap M, van der Steen P, Foppen JWA, Lens PNL, 2014. Quantification of microbial risks to human health caused by waterborne viruses and bacteria in an urban slum. *J Appl Microbiol 116*: 447-463.
24. Juberg DR, Alfano K, Coughlin RJ, Thompson KM, 2001. An observational study of object mouthing behavior by young children. *Pediatrics 107*: 135-142.
25. Jamieson RC, Gordon RJ, Sharples KE, Stratton GW, Madani A, 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Can Biosyst Eng* 44: 1-9.
26. Savichtcheva O, Okayama N, Okabe S, 2007. Relationships between Bacteroides 16S rRNA genetic markers and presence of bacterial enteric pathogens and conventional fecal indicators. *Water Res 41*: 3615-3628.
27. Howard G, Pedley S, Tibatemwa S, 2006. Quantitative microbial risk assessment to estimate health risks attributable to water supply: can the technique be applied in developing countries with limited data? *J Water Health 4*: 49-65.
28. Humphrey JH, Jones AD, Manges A; SHINE Trial Team, 2015. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial: Rationale, Design, and Methods. *Clin Infect Dis 61*: S685-S702.
29. Grusec JE, Danyliuk T, 2014. Parents' attitudes and beliefs: Their impact on children's development. *Encyclopedia on early childhood development*. Montreal, Quebec: Centre of Excellence for Early Childhood Development and Strategic Knowledge Cluster on Early Child Development.

CHAPTER 6: CHILD SOIL INGESTION: FREQUENCY, RELATIONSHIP WITH HOUSEHOLD FLOOR MATERIAL, CAREGIVER PERCEPTIONS, AND ASSOCIATIONS WITH CHILD DIARRHEA IN RURAL GHANA[**]

6.1 Abstract

Objectives: The objectives of this work were to evaluate the prevalence, frequency, and amount of soil children ingested, if household flooring material in the bedroom (earth versus concrete) affected soil ingestion characteristics, if soil ingestion was associated with diarrhea independent of household floor type, and how caregivers perceived the act of their children ingesting soil.

Methods: We conducted household surveys with 309 households in northern Ghana, which included 526 children under five, with 247 children aged 6-36 months. We also collected soil samples from a subset of 31 households to measure levels of fecal contamination.

Results: Among all children, 15% were reported to have ingested soil in the past week, including 28% of children aged 6-36 months. Among children reported to have ingested soil, the median ingestion frequency was 14 times in the past week, and the median amount of soil ingested each time was half a handful. Approximately 85% of caregivers whose children ingested soil in the past week reported they thought it was unsafe and were more likely to report stopping their child from ingesting soil, but these responses did not affect the quantity of soil ingested in the past week. After adjusting for household floor material and other potential confounding variables, soil ingestion was associated with diarrhea for all children under five [adjusted odds ratio (adj. OR)=3.12, 95% confidence interval (CI) 2.21-4.40], and for children aged 6-36 months (adj.

[**] This chapter is in preparation: Bauza, V., Byrne, D.M., Trimmer, J.T., Lardizabal, A., Atiim, P., Asigbee, M. A. K., Guest, J.S. Child soil ingestion: Frequency, relationship with household floor material, caregiver perceptions, and associations with child diarrhea in rural Ghana. To be submitted to *Tropical Medicine & International Health.*

OR=2.14, 95% CI 1.63-2.80). There was no association between household floor material and whether or not the caregiver observed the child ingesting soil.

Conclusions: While improving floor material may also help to reduce diarrhea, our results suggest soil ingestion is associated with diarrhea independent of household floor material, and separate interventions may be necessary to prevent exploratory soil ingestion.

6.2 Introduction

A growing number of studies of young children in low-income countries have observed a link between exploratory soil ingestion and negative health consequences [e.g., diarrhea,[1–3] environmental enteric dysfunction (EED),[4] stunting[4]], suggesting that soil ingestion may be a potentially important fecal exposure route. These health consequences have substantial effects on child health and survival globally. Diarrhea is the second leading cause of death in children under five, responsible for an estimated 525,000 deaths each year.[5] EED is also believed to be widespread in low-income countries and a possible underlying condition of stunting.[6] Stunting affects over one-fourth of children in developing countries and is estimated to be the underlying cause of approximately 15% of deaths in children under five.[7] Given the severity of the health consequences with which exploratory soil ingestion may be associated, its further characterization as a potentially important fecal exposure route in children is warranted.

Intentional soil ingestion is often referred to as geophagy; however, much of the literature regarding geophagy refers to older children and adults who may intentionally ingest soil in a manner involving the specific selection of soils to ingest that may be sold in markets, cooked, or otherwise prepared before consumption,[8] and therefore may not be applicable to exploratory soil

ingestion as part of young children's mouthing behavior. The few structured observation studies focused on young children in low-income countries have found high levels of mouthing behavior and soil ingestion. In rural Bangladesh, 28% of children 6-12 months and 35% of children 12-18 months old were observed to put soil in their mouths during a 5-hour observation period.[9] A separate study in rural Bangladesh observed 18% of children 6-30 months putting soil in their mouths and over 82% of children mouthing either soil or an object or food contaminated with soil during a 5-hour observation period.[10] Similarly, in rural Zimbabwe, 15% of the children were observed to ingest soil during a 6-hour period.[11] The high frequency of these activities over a short time demonstrates the potential exposure risk and the need for a better understanding of the factors affecting soil ingestion. Additionally, although these provide estimates of soil ingestion by young children over 5- to 6-hour structured observation periods, little is understood about the prevalence and frequency of soil ingestion over longer periods of time (e.g., over the course of a week) or in children 30 months to five years old, both of which are important for estimating children's continued exposure risk from this activity.

One factor that could be related to the prevalence or frequency of soil ingestion is the presence of an earth floor in households, which has been previously associated with a higher risk of diarrhea.[12,13] Although not yet directly studied, it might be assumed that floor upgrades could reduce the prevalence and frequency of soil ingestion, since a child with a finished floor in their household would no longer be exposed to soil within their home. However, a recent study in Kenya found a high prevalence of soil ingestion among children with finished household floors, likely due to children spending large amounts of time outside,[3] suggesting that soil ingestion could remain an important exposure route for children living in households with finished floors.

However, more research is needed to understand if finished flooring in households reduces the prevalence or frequency of soil ingestion compared to earth flooring, which would provide insight into whether floor upgrades are an effective means of reducing soil ingestion.

The overarching goal of this work was to gain a better understanding of the characteristics of child soil ingestion, whether they vary based on household floor material, and the implications for child health among children under five years old in rural Ghana. The specific objectives were to evaluate (i) if household flooring in the bedroom (earth versus finished) affected the prevalence, frequency, or amount of soil ingested, (ii) if soil ingestion was associated with diarrhea independent of household floor type, (iii) what the prevalence and frequency of soil ingestion was in children under five over the course of a week, and (iv) how caregivers perceived the act of their children ingesting soil. We conducted household surveys with 309 households in northern Ghana, which included 526 children under five, of which 247 were children aged 6-36 months. We also collected soil samples from a subset of 31 households to measure levels of fecal contamination. A better understanding of these factors could inform future WASH interventions by providing more details on the characteristics and frequency of soil ingestion, as well as an improved understanding of whether exploratory soil ingestion is likely to be reduced by floor upgrades or if separate interventions may be necessary.

6.3 Methods

Study site and sampling frame

This study uses cross-sectional data for 526 children under five, including 247 children aged 6-36 months, from 309 households in six villages of northern Ghana. The household surveys were

conducted in January 2017 as part of a baseline survey for a longitudinal study characterizing risks of exposure to fertilizer, pesticide, and fecal contamination. The six villages included are part of the United States Agency for International Development's (USAID's) Soybean Innovation Lab (SIL) field sites in Northern Ghana: three villages from Tolon District (Kpalsagu, Chirifoyili, Yipelgu) and three villages in Karaga District (Kuduli, Shebo, Nyensagba). Each village was divided into enumeration areas, and compounds within each enumeration area were randomly selected and approached to determine if they contained any households with children under five years old. Households with children under five within one compound were then systematically selected (by approaching everything fifth household moving clockwise from the compound entrance) for inclusion, with a maximum of three households included per compound. The villages in Tolon District have previously received soybean intervention packets from SIL, which included items such as soybean seeds, fertilizer, and herbicides, whereas the villages in Karaga serve as control villages for other SIL studies. Soil samples were also collected from a subset of 31 households for *Escherichia coli* and total coliform enumeration.

Ethical approval

Informed consent was obtained from the household's primary caregiver prior to enrollment and the study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

Household surveys

Household surveys were conducted in the local language by trained Ghanaian enumerators to collect information about household demographics, child health, and soil ingestion

characteristics. In this study, diarrhea was defined as three or more bowel movements within a 24-hour period. Caregivers were asked to report if their child had diarrhea within the past 2 days or within the past 7 days. With reference to soil ingestion, caregivers were asked, "Have you observed this child putting soil, mud, clay, or sand into his or her mouth in the past 7 days?" and, if the answer was yes, then "How many times in the past 7 days did you observe your child putting soil, mud, clay, or sand into his or her mouth?", "About how much soil, mud, clay, or sand did you watch your child put into his or her mouth each time?", "Did you stop your child from putting soil, mud, clay, or sand into his or her mouth when you saw him or her do it?", and "Do you think it is safe for your child to put soil, mud, clay, or sand into his or her mouth?" Although caregiver reporting of events does not provide the same level of detail as structured observation, caregiver observed soil ingestion was found to agree well with soil ingestion events noted during structured observation in a previous study in Bangladesh.[4]

Soil Sampling and *E. coli* and Total Coliform Enumeration

For a subset of 31 households, soil samples were collected from inside each compound (where applicable) or from the compound entrance for each household, following the methods of Bauza et al.[3] Approximately 10 grams of soil was collected from the ground surface using sterile polystyrene sampling spoons (Nasco, Fort Atkinson, WI) from an area approximately 10 cm by 10 cm. Each soil sample was placed in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI), and stored in a cooler with ice packs for up to 7 hours before being processed in a laboratory. During sample collection, the relative humidity was 10% and the temperature ranged from 27-43^{o}C. The sampled households included 15 households in the Shebo and Nyensagba villages of Karaga and 16 households in the Kpalisagu and Chirifoyili villages in Tolon.

Following a method slightly modified from Boehm et al.[14] to recover *E. coli* from sand samples, each soil sample was homogenized by hand. Next, 2 grams was hand shaken in 20 ml of sterile deionized (DI) water for 2 minutes and allowed to settle for 30 seconds. The supernatant was then diluted and *E. coli* and total coliforms were enumerated using Colisure media (IDEXX, Westbrook, ME) and Quanti-tray/2000 (IDEXX, Westbrook, ME) materials and the Most Probable Number (MPN) method. Trays were incubated at 35^{o}C for 24-48 hours following manufacturer's guidelines. Blanks of the DI water used for dilution were processed daily, and all were negative for *E. coli* and total coliform. In addition, 5 grams of each sample was oven-dried at 105^{o}C for 24 hours to measure soil moisture content.

Statistical Analysis

All statistical analysis was performed with Stata version 13.1 (StataCorp LP, College Station, TX). Chi-square tests were used to test for differences in dichotomous outcomes for dichotomous dependent variables (e.g., if caregivers who viewed soil ingestion as unsafe were more likely to report stopping their child from ingesting soil). T-tests were used to test for differences in continuous variable outcomes (e.g., *E. coli* and total coliform MPN, estimated soil quantity consumed) between dichotomous variable categories, such as district. Logistic regression was used to evaluate associations between reported child diarrhea and child soil ingestion. The variables of child age, whether the household owns a bed, whether the household has electricity, whether the household has an earth or concrete floor in the bedroom, whether the household owned poultry, the education level of the female caregiver, the education level of the male caregiver, whether the household had access to an improved water source, whether the household

uses a toilet, and whether the caregiver reported washing his/her hands with soap before feeding a child were included as potential confounding variables in the adjusted logistic regression model. Standard errors were clustered by village in the regression model to account for sampling strategy.

The quantity of soil ingested by children provides more detailed exposure information and was estimated based on survey responses (Table 6.1) and past work measuring soil in young children's hands. Children reported to ingest the amount of soil normally on their fingers were assigned an estimated quantity of 0.25 g per event, based on the estimation of Ngure et al. that this was the amount that made 2-3 fingers visibly dirty for a one year old.[11] We assigned the value of 2.5 g to children reported to consume a handful of soil. This estimate was also based the on the results of Ngure et al., who found that a one-year could hold approximately 2.5 grams of soil in his/her hand without spilling.[11] We further estimated half a handful as 1.25 g, the amount between two fingers as 0.5 g, and a dry lump of clay at 0.5 g. For our estimations, these values were assumed to be the same regardless of age, even though hand size would be likely to increase with age.

6.4 Results

Household characteristics

This study included 309 households located in Karaga and Tolon Districts in northern Ghana, with each district accounting for approximately half of the total households and children (153 households with 258 children from Karaga and 156 households with 268 children from Tolon). Household characteristics that are similar for both districts are reported for all study participants,

whereas differing characteristics are reported by district. Households had a mean of 4.5 members [standard deviation (SD) 3.8], including a mean of 2.1 children under five (SD 1.3). The education level attained by adults in our study was low, with 83.5% of primary female caregivers (N=258) reporting they had received no formal schooling. Additionally, farming was widespread, with 98.7% of households (N=305) reporting they had engaged in agricultural activities in the past year and 79.6% (N=242) reporting livestock ownership, including 73.7% (N=224) households owning poultry.

Although open defecation was common in both districts, households in Karaga had a higher level of access to improved sanitation and water infrastructure than households in Tolon. In Karaga, 56.7% of households (N=87) reported practicing open defecation, 36.6% reported using a pit latrine with slab (N=56), and 6.5% reported using a pit latrine without slab (N=10). However, in Tolon, 91.7% of households reported practicing open defecation (N=143), with only 7.7% of households (N=12) using a ventilated improved pit (VIP) latrine or a pit latrine with a slab, and 0.6% of households (N=1) using a pit latrine without a slab. More households in Karaga also had access to an improved water source. In Karaga, 75.8% of households (N=116) used a borehole as their primary drinking water source, 23.5% (N=36) used a shallow dug well or tubewell, and 0.7% (N=1) used surface water. However, in Tolon 93.6% of households (N=146) used surface water as their primary drinking water source, with the remaining households getting their water from a tap, shallow dug well, or a water vendor.

Soil ingestion: prevalence, frequency, and estimated quantity

For each child, caregivers were asked to report if they had seen their child put soil in his/her mouth, how many times in the past week they had seen the child put soil into his/her mouth, and the approximate the amount of soil each time. Across age groups, the prevalence, frequency, and quantity of soil ingested per event varied (Table 6.2). The highest prevalence of soil ingestion was reported for children between 12-24 months old at 38%, followed by children 6-12 months old at 27%, and children 24-36 months old at 19%. The prevalence of soil ingestion was lower for children 36-48 months at 8%, for children 48-60 months at 3%, and no children under the age of 6 months were reported to have ingested soil.

Across all children included in the study, the prevalence of soil ingestion in the past week was 15% for children under five (N=80) and 28% for children aged 6-36 months (N=69). Among children reported to have ingested soil, the median ingestion frequency was 14 times in the past week, and the median amount of soil ingested each time increased to half a handful. Although it was not explicitly asked as part of the survey, discussions with enumerators about survey responses indicated that typically when 7, 14, or 21 times per week was reported on the survey, this indicated that the caregiver reported observing soil ingestion once per day, twice per day, or three times per day in the past week (reported for 16.1%, 21.0%, and 22.2% of children, respectively). Some caregivers also responded “yes” when asked if they had observed their child ingesting soil, but when asked for the amount of soil ingested, they reported that it was the amount of dirt normally on their child’s hands (Table 6.1). For these children, we assumed that the activity reported as soil ingestion was indirect soil ingestion through hand to mouth contact instead of intentional soil ingestion. Unless otherwise noted, we have not counted these children

as participating in soil ingestion. Due to high levels of hand to mouth contact reported for other young children in observation studies,[9–11] we assume this type of indirect soil ingestion is likely occurring in many of the children included in our analysis, even when it has not been reported as soil ingestion by caregivers. However, if we did include children reported to ingest the amount of soil normally on their hands as soil ingestion, then 19% (N=100) of children under five would have been reported to have ingested soil in the past week, including 35% of children aged 6-36 months (N=87).

Soil ingestion: caregiver perceptions

Approximately 85% of caregivers whose children ingested soil in the past week reported they thought it was unsafe. These caregivers were more likely to report stopping their child from ingesting soil (χ^2=7.41, p=0.006), with 82.7% reporting they stopped their child from ingesting soil. However, there was no difference in the amount of soil consumed in the past week between children of caregivers who reported stopping soil ingestion and those who reported not stopping it (mean of 15.1 g of soil consumed for children stopped versus 16.7 g for children not stopped, t=0.44, p=0.66), as estimated by assigning quantities to the amount of soil (e.g., half a handful) that caregivers reported their child had consumed (explained in detail in the Methods section).

Soil ingestion: associations with diarrhea

The prevalence of diarrhea was high, with diarrhea in the past week reported for 29% of children under five (Table 6.3). After correcting for household floor material and other potential confounding variables, soil ingestion was associated with more than triple the odds of diarrhea for all children under five (adjusted odds ratio [adj. OR]= 3.12, 95% confidence interval [CI] 2.21-4.40; Table 6.4), and more than double the odds of diarrhea for children aged 6-36 months

(adj. OR=2.14, 95% CI 1.63-2.80, Table 6.4). The analysis was also rerun stratifying by Karaga and Tolon Districts, and the relationship between soil ingestion and diarrhea remained significant for each district.

Among all children reported to have consumed soil, including children who consumed the amount normally on their fingers, increased quantity of soil ingested was associated with increased prevalence of diarrhea, both for the quantity of soil ingested at each ingestion event (mean of 0.88 g per event for children without diarrhea versus 1.23 g for children with diarrhea, t=-2.28, p=0.013) as well as the total amount ingested in the past week (mean of 10.6 g per week for children without diarrhea versus 14.9 g for children with diarrhea, t=-1.72, p=0.045). The frequency of soil ingestion among children reported to consume soil was not associated with diarrhea (mean of 12.7 ingestion events for children without diarrhea versus 12.1 for children with diarrhea, t=0.41, p=0.680). However, when the amount normally found on fingers was excluded as soil ingestion, there was only marginal evidence for an association between increased quantity of soil ingested per event and increased prevalence of diarrhea (mean of 1.13 g per event for children without diarrhea versus 1.38 g for children with diarrhea, t=-1.46, p=0.074). If we assumed that all children not reported to have been seen ingesting soil had indirectly ingested 0.25 grams per day from hand to mouth contact, then the association between quantity of soil ingested and diarrhea was significant, both for the quantity of soil ingested at each ingestion event (mean of 0.34 g per event for children without diarrhea versus 0.57 g for children with diarrhea, t=-4.02, p<0.0001) as well as the amount of soil ingested in the past week (mean of 3.0 g per week for children without diarrhea versus 5.9 g for children with diarrhea, t=-3.47, p=0.0003).

Blood in stool in the past seven days was also reported for 34 (6.4%) of children. There was an association between soil ingestion and blood in stool (adjusted OR=2.38 95% CI 1.29-4.42) for children under five, but not for children 6-36 months old (adjusted OR=1.02 95% CI 0.30-3.53).

Soil ingestion and diarrhea: relationships with floor material

The majority of children lived in households that had a concrete floor in the bedroom, but 7.4% of children lived in households with earth floors (N=39). Although there was evidence that having an earth bedroom floor was associated with diarrhea (χ^2=6.38, p=0.01), there was no evidence that having an earth bedroom floor was associated with soil ingestion (χ^2=0.78, p=0.38). There was also no association between floor material and frequency of soil ingestion (mean of 12.2 ingestion events for children with concrete floors versus 12.3 for children with earth floors, t=-0.04, p=0.97) or between floor material and quantity of soil consumed over a week (mean of 14.7 g per week for concrete floors versus 22.8 g for earth floors, t=-1.04, p=0.369). The results of the adjusted logistic regression model offer further evidence that floor material and soil ingestion are both independently associated with diarrhea (Table 6.4) as soil ingestion remains significant in the adjusted model that includes a variable for bedroom floor material.

Fecal contamination in soil

E. coli was detected above the limit of detection (LOD, approximately 100 MPN per gram dry soil) in 45% of soil samples (N=14), and total coliforms were detected above the same LOD in 74% of soil samples (N=23). After log-transforming the count data and assigning half the LOD

to negative samples, the log-transformed mean *E. coli* MPN was 2.17 (SD 0.70, range 1.68-4.23) and the log-transformed mean total coliform MPN was 3.27 (SD 1.26, range 1.69-5.40). However, our ability to accurately estimate the MPN of *E. coli* and total coliform in soil samples is hindered by the large proportion of samples that were below the LOD. Soil moisture content was also low for all samples, ranging from 0.2-3.43% (mean=1.2, SD 0.62). There was no difference among the detection of *E. coli* or total coliforms for samples taken from shade, partial sunlight, or full sunlight. The MPN count for *E. coli* and total coliforms were higher for samples taken from Tolon than Karaga (log-transformed mean of 1.91 for Karaga and 2.41 for Tolon for *E. coli*, t=-2.1, p=0.023; log-transformed mean of 2.82 for Karaga and 3.67 for Tolon for total coliform, t=-1.98, p=0.030).

6.5 Discussion

This work found evidence that child soil ingestion was associated with diarrhea independent of whether the household had an earth floor, that children who ingest soil often ingest it frequently during a week, and that a caregiver's perception of soil ingestion as unsafe may not reduce the amount of soil her/his children ingest in this rural Ghanaian setting. Along with adding to the growing body of evidence that soil ingestion may be an important pathway for fecal contamination that should be considered in holistic water, sanitation, and hygiene (WASH) interventions,[3,4,11] this study adds detailed knowledge about the prevalence, weekly frequency of soil ingestion, and estimated quantities of ingestion for over 500 children in northern Ghana, including data for older children under 5 (most past studies related to soil ingestion prevalence and frequency in children in low-income countries have only included children up to 18 months[9,11] or 30 months[4]).

We found the highest prevalence of soil ingestion among children aged 12-24 months, which is consistent with two previous structured observation studies in Bangladesh – one observing the number of times children put soil in their mouths,[9] and one observing the number of times children put soil in their mouth or had any soil contact.[10] However, our study found that soil ingestion was observed in 36% of children aged 24-30 months, illustrating that it may be important to include children over 18 months in future soil ingestion studies, even if these children might have lower frequencies of hand to mouth or object to mouth contact. No soil ingestion events were reported for children under 6 months, which is consistent with a previous study[3] and may be due to these children spending more time indoors or off of the ground. A previous study in urban Ghana found that children under one year old spent most of their time playing or sleeping off the ground, compared to older children.[15]

While structured observation studies typically try to quantify soil ingestion events over 5- to 6-hour observation periods (which is useful for exposure estimates), we investigated the frequency of soil ingestion over the course of 7 days to better understand daily and weekly patterns of soil ingestion. It was common for caregivers to report they had observed their child ingest soil 7 times, 14 times, or 21 times in the past week (i.e., likely responses of once, twice, or three times daily, as described by enumerators), with only 30% of caregivers who observed soil ingestion reporting that a child had consumed soil less than 7 times in the past week. These results indicate soil ingestion is occurring very frequently, and soil ingestion frequency may have a consistent pattern from one day to the next. However, future studies would be necessary to confirm the day-to-day pattern.

We also found evidence for an association between soil ingestion and diarrhea. Additionally, while the quantity of soil reported to be ingested was associated with increased prevalence of diarrhea, soil ingestion frequency was not, suggesting that the quantity of soil ingested was more important for exposure than the number of hand-to-mouth contacts and that the observed association between soil ingestion and increased diarrhea prevalence is not only due to increased hand-to-mouth activity among these children. However, the ingestion of poultry feces was not considered as part of this study, which may also be linked with diarrhea from similar exploratory behaviors of ingesting items from the environment. Poultry ownership was common in these households, with approximately three-quarters of children living in households with poultry. Past studies have observed children consuming poultry feces in low-income countries[1,11] and found an association between poultry feces consumption and diarrhea.[1] Although the possible ingestion of poultry feces by children was not included in our study, we did attempt to account for this by controlling for poultry ownership as a potential confounding variable in our adjusted logistic model.

There was also no association between household floor material and whether or not the caregiver observed the child ingesting soil, the frequency at which a child ingested soil, or the quantity of soil consumed over the past week, suggesting that soil ingestion is associated with diarrhea independent of household floor material. While there was evidence that improving floor material may also help to reduce diarrhea, our results suggest that separate interventions may still be necessary to prevent exploratory soil ingestion. However, a past structured observation study in rural Bangladesh including households with earth floors found that the frequency of soil

ingestion was independent of whether children were inside or outside of the household.[9] It is possible that children included in our study from households with finished floors may actually have ingested soil less frequently than those with earth floors, but caregiver reporting of soil ingestion frequency may not have been sensitive enough to observe this difference. However, the association seen between soil ingestion and diarrhea in our logistic regression model that was independent of floor material might indicate soil ingestion is occurring at a high enough frequency outside of households that infectious doses of diarrhea-causing pathogens may be reached even if the child is not consuming soil inside the household. This is consistent with a past study in Kenya that found an association between soil ingestion and child diarrhea, despite high coverage rates of finished floor among households.[3]

The evidence from this study suggests soil ingestion is a frequent behavior in young children that could lead to diarrhea. As such, new approaches to WASH interventions that incorporate methods for reducing soil ingestion are needed. Our results suggest it may be necessary to move beyond targeted education of caregivers about the potential health consequences of soil ingestion. About 85% of caregivers in our study reported they thought soil ingestion was unsafe, which was associated with caregivers reporting they stopped their child from consuming soil when they saw the event happen. However, despite these reports, there was no difference in the quantity of soil ingested by children whose caregivers reported stopping them and children whose caregivers did not. We asked caregivers whether they stopped their child from ingesting soil prior to asking them if they thought soil ingestion was safe to avoid potential over-reporting. If a prior question implied that soil ingestion was unsafe and that stopping their child was the correct thing to do, caregivers may have been more likely to respond affirmatively. However, the

lack of a difference in soil ingestion quantity may still be due to reporting bias, as a past study found that more caregivers reported stopping their child from ingesting soil than were actually observed to do so during structured observation.[4] Regardless, it illustrates that education interventions targeted toward soil ingestion safety may be insufficient for reducing soil ingestion. Rather, education about specific methods to reduce soil ingestion should be incorporated. More comprehensive interventions may also be necessary, such as the hygienic portable play spaces that are being evaluated in the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial,[16] to effectively reduce children's fecal exposure through soil ingestion.

6.6 Acknowledgements

The authors thank David Sombie and Emmanuel Osei-Mensah at Catholic Relief Services for their help training enumerators and coordinating fieldwork, the enumerators for conducting household interviews, and the village leaders and participating households who made this study possible. We also thank Dr. Stephen Nutsugah, Dr. Roger Kanton, Dr. Askia Mohammed, and Prosper Amenuvor at Savanna Agricultural Research Institute for providing laboratory space and assistance. This work and Amanda Lardizabal were funded by the United States Agency for International Development (USAID) as part of the Soybean Innovation Lab. Valerie Bauza was supported by the National Science Foundation Graduate Research Fellowship Program under grant no. DGE-1144245, Diana Byrne was funded by the Department of Civil and Environmental Engineering at UIUC, and John Trimmer was funded by the Illinois Distinguished Fellowship at UIUC.

Table 6.1. Reported amount and estimated mass of soil ingestion per ingestion event for children under five years old.

Amount Ingested	Number of Children (N, %)	Estimated quantity (grams)
Amount of dirt normally on fingers*	20 (20%)	0.25
Small lump of dry soil/clay	2 (2%)	0.50
Amount he/she could hold between two fingers	29 (29%)	0.50
Half of a handful	30 (30%)	1.25
A handful	19 (19%)	2.50

*Children reported to ingest the amount of soil normally on fingers have been classified as no soil ingestion throughout this paper unless otherwise noted, as this indirect soil ingestion is assumed to be a part of hand to mouth activities and not intentional ingestion of soil.

Table 6.2. Prevalence, frequency, and estimated quantity of soil ingestion for children under five years old.

	Total (N)	Reported Soil Ingestion (N, % of Age Group)	Frequency of soil ingestion per week (mean, SD)	Estimated Quantity (grams) of soil ingested per event (mean, SD)
All children included in study	526	80 (15.2%)	12.2 (7.8)	1.26 (0.77)
Children 0 to <6 months	55	0 (0%)	0	0
Children 6 to <12 months	49	13 (26.5%)	15.2 (10.3)	0.85 (0.39)
Children 12 to <24 months	98	37 (37.8%)	9.0 (6.4)	1.30 (0.85)
Children 24 to <36 months	100	19 (19.0%)	15.9 (7.1)	1.21 (0.67)
Children 36 to <48 months	104	8 (7.7%)	15.9 (4.5)	2.03 (0.65)
Children 48 to <60 months	120	3 (2.5%)	5.7 (2.3)	0.75 (0.43)

Table 6.3. Prevalence of diarrhea in children under five years old.

	Total	Reported Diarrhea (N, % of Category in Age Group)		
		All	Soil Ingestion	No Soil Ingestion
All children included in study	526	150 (28.6%)	42 (52.5%)	108 (24.2%)
Children 0 to <6 months	55	12 (21.8%)	-	12 (21.8%)
Children 6 to <12 months	49	24 (49.0%)	6 (46.2%)	18 (50.0%)
Children 12 to <24 months	98	44 (44.9%)	21 (21.4%)	23 (23.5%)
Children 24 to <36 months	100	41 (41.0%)	11 (57.9%)	30 (37.0%)
Children 36 to <48 months	104	19 (18.3%)	3 (37.5%)	16 (16.7%)
Children 48 to <60 months	120	10 (8.3%)	1 (33.3%)	9 (7.7%)

Children reported to ingest the amount of soil normally on fingers have been classified as 'no' for soil ingestion.

Table 6.4. Association between diarrhea and soil ingestion.

Child diarrhea explanatory variable	Unadjusted			Adjusted		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Children aged under 5 years (N=516)						
Soil ingestion	3.84	2.66 – 5.56	<0.001	3.12	2.21 – 4.40	<0.001
Child age				0.67	0.57 – 0.80	<0.001
Own bed				1.55	0.95 – 2.55	0.081
Has electricity				0.64	0.32 – 1.29	0.212
Bedroom floor earth				2.85	1.31 – 6.20	0.008
Owns poultry				0.73	0.38 – 1.42	0.351
Female caregiver education				1.35	0.80 – 2.28	0.257
Male caregiver education				1.41	0.83 – 2.38	0.206
Improved water source				1.21	0.46 – 3.22	0.701
Household uses toilet				1.14	0.92 – 1.41	0.229
Caregiver always washes hands with soap before feeding child				1.26	0.54 – 2.97	0.592
Constant				0.71	0.18 – 2.79	0.305
Children aged 6 months to <36 months (N=239)						
Soil ingestion	2.09	1.46 – 2.99	<0.001	2.14	1.63 – 2.80	<0.001
Child age				0.91	0.60 – 1.37	0.652
Own bed				1.52	0.75 – 3.09	0.246
Has electricity				1.05	0.41 – 2.68	0.917
Bedroom floor earth				1.75	0.61 – 5.05	0.300
Owns poultry				0.65	0.25 – 1.73	0.393
Female caregiver education				1.72	0.80 – 3.68	0.162
Male caregiver education				1.79	0.75 – 4.30	0.192
Improved water source				1.21	0.54 – 2.72	0.637
Household uses toilet				0.99	0.57 – 1.70	0.959
Caregiver always washes hands with soap before feeding child				1.31	0.58 – 2.94	0.505
Constant				0.55	0.09 – 3.24	0.505

Three “don’t know” responses for soil ingestion excluded.
Children reported to ingest the amount of soil normally on fingers have been classified as ‘no’ for soil ingestion.
Standard errors clustered by village.

6.7 References

1. Yeager, B. A., Lanata, C. F., Lazo, F., Verastegui, H. & Black, R. E. Transmission factors and socioeconomic status as determinants of diarrhoeal incidence in Lima, Peru. *J. Diarrhoeal Dis. Res.* 186–193 (1991).
2. Shivoga, W. A. & Moturi, W. N. Geophagia as a risk factor for diarrhoea. *J. Infect. Dev. Ctries.* **3,** 94–98 (2009).
3. Bauza, V., Ocharo, R. M., Nguyen, T. H. & Guest, J. S. Soil Ingestion Is Associated with Child Diarrhea in an Urban Slum of Nairobi, Kenya. *Am. J. Trop. Med. Hyg.* **96,** 569–575 (2017).
4. George, C. M. *et al.* Geophagy is associated with environmental enteropathy and stunting in children in rural Bangladesh. *Am. J. Trop. Med. Hyg.* **92,** 1117–1124 (2015).
5. World Health Organization. Diarrhoeal disease fact sheet. *WHO* (2017). Available at: http://www.who.int/mediacentre/factsheets/fs330/en/. (Accessed: 6th June 2017)
6. Humphrey, J. H. Child undernutrition, tropical enteropathy, toilets, and handwashing. *The Lancet* **374,** 1032–1035 (2009).
7. Black, R. E. *et al.* Maternal and child undernutrition and overweight in low-income and middle-income countries. *The lancet* **382,** 427–451 (2013).
8. Kutalek, R. *et al.* Geophagy and potential health implications: geohelminths, microbes and heavy metals. *Trans. R. Soc. Trop. Med. Hyg.* **104,** 787–795 (2010).
9. Kwong, L. H. *et al.* Hand-and object-mouthing of rural Bangladeshi children 3–18 months old. *Int. J. Environ. Res. Public. Health* **13,** 563 (2016).
10. Morita, T. *et al.* Mouthing of Soil Contaminated Objects is Associated with Environmental Enteropathy in Young Children. *Trop. Med. Int. Health* (2017).
11. Ngure, F. M. *et al.* Formative Research on Hygiene Behaviors and Geophagy among Infants and Young Children and Implications of Exposure to Fecal Bacteria. *Am. J. Trop. Med. Hyg.* **89,** 709–716 (2013).
12. Cattaneo, M. D., Galiani, S., Gertler, P. J., Martinez, S. & Titiunik, R. Housing, health, and happiness. *Am. Econ. J. Econ. Policy* **1,** 75–105 (2009).
13. Woldemicael, G. Diarrhoeal morbidity among young children in Eritrea: environmental and socioeconomic determinants. *J. Health Popul. Nutr. JHPN* **19,** 83–90 (2011).
14. Boehm, A. B. *et al.* Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *J. Appl. Microbiol.* **107,** 1740–1750 (2009).
15. Teunis, P. F., Reese, H. E., Null, C., Yakubu, H. & Moe, C. L. Quantifying contact with the environment: behaviors of young children in Accra, Ghana. *Am. J. Trop. Med. Hyg.* **94,** 920–931 (2016).
16. Humphrey, J. H. *et al.* The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) Trial: Rationale, Design, and Methods. *Clin. Infect. Dis.* **61,** S685–S702 (2015).

CHAPTER 7: CONCLUSIONS

7.1 Summary, Conclusions, and Contributions

Despite large investments in water and sanitation infrastructure improvements in low-income countries with goals to improve health, there is still a lack of fundamental knowledge about the relative importance of different transmission pathways and exposure points in the transmission of enteric pathogens in low-income settings. It is also not well understood what role different human and animal fecal sources play in releasing enteric pathogens to the domestic environment that then result in child exposure and illness. The research described in this dissertation aimed to provide a better understanding of these critical factors, which is crucial for designing and implementing water, sanitation, and hygiene interventions to achieve greater improvements in child health.

First, the occurrence, magnitude, and distribution of fecal contamination and four enteric pathogens (Adenovirus, *Campylobacter jejuni*, *Shigella* spp./EIEC, and *Vibrio cholerae*) that are important causes of diarrhea and gut inflammation characteristic of environmental enteric dysfunction were assessed along transmission pathways and at several common exposure points for children in a densely-populated low-income neighborhood of Nairobi, Kenya (Chapter 2). There was a high frequency of pathogen detection at several exposure points (including stored drinking water, hands, table surfaces, plate surfaces, floor surfaces, soil, standing water, open drainage ditches, and streams) despite the fact that all households that were included in the study had access to and reported that all adults in the households used a toilet or latrine.

The results also provided evidence that children were being exposed to enteric pathogens from several exposure points at the same time, that there were interactions between different transmission pathways, that ownership of chickens in this urban setting was associated with increased detection of *C. jejuni* inside households and on soil, and that *V. cholerae* was detected at several exposure points during a cholera outbreak. Furthermore, soil was found to have high levels of *C. jejuni* and *Shigella spp.*/EIEC*,* which are implicated in severe negative health consequences and could make soil an important exposure point. Prior to this study, there was very limited data measuring pathogens for many of these exposure points and few to no studies that looked at these specific enteric pathogens at these common exposure points in an urban slum setting, despite their importance for disease transmission.

Next, new methods were developed to better understand the human sources of fecal contamination that were leading to the high levels of fecal contamination and enteric pathogens measured in the domestic environment. Since all households included in the study already had access to a toilet/latrine used by adults, it was investigated whether feces from young children who were not old enough to use toilets might be contributing to the household fecal contamination levels. In order to do this, a method to discern between human feces from young children versus human feces from older children/adults needed to be developed. Methods were developed to discern between these two sources using bacterial community sequencing, validated in a spiking study, and found to perform well for identifying the dominant source of human fecal contamination in a given sample as well as the presence/absence of each source (Chapter 3). The observation- and survey-independent experimental technique developed to understand the prevalence and distribution of fecal contamination from young children in the domestic

environment will be valuable for future WASH studies as a way to better characterize the sources of human fecal contamination at key exposure points, better target interventions, and better assess the performance of sanitation interventions.

After method development, the dominant source and presence/absence of human feces from young children versus older children/adults at critical exposure points inside and outside the household environment were evaluated. Human feces from young children dominated samples taken from the indoor environment (caregiver and child hands, tables, plates) and human feces from older children and adults tended to dominated samples taken from the outdoor environment (standing water, streams), while both were dominant in open drainage ditch samples (Chapter 3). This work provided strong evidence that young children's feces, a source that is commonly overlooked and not eliminated by toilet/latrine installation, is an important contributor to household fecal contamination.

After identifying young children's feces as an importance contamination source in Kenya, the larger implications of child feces disposal practices on child health were assessed using Demographic and Health Survey data from 34 low- and middle-income countries (Chapter 4). Improved child feces disposal practices, which required disposal of child feces into an improved toilet, were strongly associated with reductions in stunting as well as improvements in other undernutrition outcomes, serving as the first study to provide a link between child feces disposal practices and stunting. Although the benefits were not as substantial as disposal in an improved toilet, child growth benefits were also found to be associated with child feces disposal in an unimproved toilet. These findings have critical policy implications, as they indicate that there is

potential for greater improvements in child health to be realized from the promotion of hygienic child feces disposal practices after installation of toilets.

Chapters 5 and 6 continued the evaluation the health consequences linked with exposure from commonly ignored sources or pathways, by analyzing the association between child diarrhea and soil ingestion. Strong associations were found between soil ingestion and child diarrhea in an urban slum setting in Kenya (Chapter 5) and a rural setting in northern Ghana (Chapter 6), despite high levels of finished floor in households in both settings. There was a high prevalence of soil ingestion in both settings, indicating this is likely a common exposure pathway for children in low-income countries. Detailed soil ingestion frequency and quantity information collected in Ghana also demonstrated the high frequency of soil ingestion, and that the amount of soil consumed was a better predictor of diarrhea than frequency of consumption. Finally, although most caregivers perceived the act of soil ingestion as an unsafe activity that they reportedly stopped their child from doing, there was no difference in the quantity of soil consumed for children that had caregivers that reported stopping ingestion compared to children with caregivers that did not stop their children, indicating that interventions that go beyond education about the potential dangers of children consuming soil are needed to reduce exploratory soil ingestion by children.

Taken together, this work identified high levels of enteric pathogen contamination at numerous indoor and outdoor exposure points in an urban slum environment, and performed detailed investigations of young children's feces as a contamination source and soil ingestion as an exposure point. The detection and quantification of pathogens along multiple exposure pathways,

the development of a experimental method for microbial source tracking of young children feces, the identification of young children's feces as a dominant human fecal contamination source in households, the evaluation of a link between child feces disposal practices and child stunting, the characterization of child soil ingestion practices, and the evaluation of a link between soil ingestion and diarrhea all advance knowledge related to fecal contamination exposure and transmission in low-income countries, and ultimately can be used to better prioritize and improve water, sanitation, and hygiene interventions.

7.2 Future Research Directions

Sanitation interventions that aim to increase access to toilets/latrines have often been found to not reduce fecal contamination levels along transmission pathways. However, these interventions are often designed without components that improve the management of child feces, which may continue to contaminate the environment after the installation of a toilet or latrine. Although low levels of sanitation coverage (or use) and animal feces may contribute to the lack of reductions in fecal contamination at domestic exposure points, contamination from young children's feces may also play an important role in the levels of fecal contamination measured after a sanitation intervention. The microbial source tracking techniques developed in this dissertation could be used at exposure points before and after sanitation interventions to evaluate the efficacy of toilet/latrine installation for reducing contamination from young children's feces, as well as evaluate how the relative contribution of fecal contamination from young children and older children/adults changes after the intervention.

The research in this dissertation also found high levels of enteric pathogen transmission at several exposure points in an urban slum setting in Kenya. However, not all enteric pathogens were measured and the distribution of enteric pathogens is likely to vary from one site to another, which should influence intervention recommendations for specific sites. Experimental techniques such as TaqMan array cards or microfluidic PCR that enable the quantification of multiple enteric pathogens at the same time could be used in future studies to provide a greater level of detail about the distribution of enteric pathogens along multiple pathways. However, these methods are expensive, and are often not feasible in low-income settings. Therefore, there is a need for future research to focus on the development of rapid, low-cost detection methods for pathogens that can be performed in field settings. These types of methods would enable rapid characterization of transmission in households and communities and could enable better assessments of interventions. Additionally, more assessments of pathogens in multiple settings could enable models to be developed that predict the greatest points of pathogen exposure based on site-specific characteristics.

The enteric pathogen information generated as a part of this dissertation could also be used, in combination with exposure studies in similar settings, to inform future QMRA models that could provide better guidance for the relative importance of different transmission pathways in disease transmission. Currently, there are few comprehensive QMRAs in low-income countries and most are based on fecal indicator bacteria, which may not always correlate well with actual pathogens.

Additionally, the research in this dissertation demonstrated that contamination from young children's feces is common inside of households and that child feces disposal practices are

associated with negative health outcomes, but this work did not assess methods to improve child feces disposal. Future research is needed to find effective methods of improving child feces management practices. As discussed in Chapter 4, there have been a few interventions that have tried to improve this behavior, but many have been unsuccessful at achieving sustained improvements in child feces management. Future interventions should consider user-centered design and bottom-up innovation processes to develop better enabling products for child feces management and integrate these products with education. Engaging with local entrepreneurs to develop and market enabling products for child feces management may also improve the long-term sustainability of an intervention.

Finally, future research is also needed to better understand soil ingestion and develop interventions that could reduce this practice in highly contaminated environments. Structured observation studies over longitudinal timescales could better inform child exposure to soil by understanding if soil ingestion changes from day to day, or if similar patterns of ingestion are common within the same child over longer timescales. Additionally, the research in this dissertation suggested that education aimed at convincing caregivers that soil ingestion is not good for their children is unlikely to be effective at reducing child soil ingestion on its own, as many caregivers of children who consumed soil reported that they already believed this action was unsafe. Portable play spaces are currently being tested as part of the SHINE trial, but additional interventions should also be explored. The use of pacifiers or other similar objects could be explored as a method to provide an alternative to children mouthing soil and other soil-contamination objects in their environment and could potentially reduce soil consumption, as long as pacifiers are cleaned adequately before being put in children's mouths and after they are

dropped on the ground. Overall, there are several different sources of fecal contamination and many exposure points in children's domestic environments that are likely important in disease transmission. Therefore, a portfolio of solutions that target reducing enteric pathogen transmission along multiple different pathways is likely needed to achieve the greatest child health benefits from interventions.

APPENDIX A: SUPPLEMENTAL INFORMATION FOR CHAPTER 2

ENTERIC PATHOGENS FROM WATER, HANDS, SURFACE, SOIL, DRAINAGE DITCH, AND STREAM EXPOSURE POINTS IN A LOW-INCOME NEIGHBORHOOD OF NAIROBI, KENYA

A.1 Methods

Quality assurance and control

E. coli. At least one filtration blank was processed daily and approximately 10% of samples were processed in duplicate. All filtration blanks were negative and duplicate samples were generally in agreement.

qPCR. Every qPCR assay included four extraction blanks from different extraction kits and each plate include three no template controls. All extraction and no template control blanks were negative, with the exception of one no template control sample on the *Vibrio cholerae* plate that was slightly positive. However, since six of the seven blanks were negative, and all no template controls were negative of the inhibition plate run with *V. cholerae,* it is expected that this error was not due to reagent contamination or no-specific amplification and did not affect the other results reported.

The linearity (R^2) and efficiency (e) of all qPCR assays were within acceptable ranges. For Adenovirus R^2=0.998 and e=96%, for *Campylobacter jejuni* R^2=0.999 and e=107%, for *Shigella* spp./EIEC R^2=0.999 and e=98%, and for *V. cholerae* R^2=0.998 and e=94%.

Inhibition was assessed for at least 10% of samples from each sample types using the *V. cholerae* assay, using a linearity of response method similar to the method described in Cao et al.[1] All samples were spiked with 3×10^3 gene copies/µl of the target gene DNA prior to completing dilutions. For soil samples, linearity was assessed for two 5-fold dilutions (1:5 and 1:25) of the DNA extract. For all other sample types, linearity was assessed for a ten-fold (1:10) dilution. For the five-fold dilutions, samples with a ΔC_q less than 1.32 were considered inhibited and for the ten-fold dilutions, samples with a ΔC_q less than 2.32 were considered inhibited. For each dilution the ΔC_q is one cycle less than the expected amplification value assuming an efficiency of 100%, a technique recommended by Cao et al to account for possible pipetting error when assessing for inhibition.[1] We found no evidence of inhibition in samples from drainage ditches, standing water, floor, tables, plates, hands, or source water samples. Although five of the household water samples tested showed no evidence of inhibition (83% of total samples tested), one of the household water sample tested had a minor inhibition that was lower than one order of magnitude difference (ΔC_q=1.97). The stream sample tested also showed evidence of very minor levels of inhibition (ΔC_q=2.22). Five of the six soil samples tested for inhibition (83%) showed minor levels of inhibition between the 1:5 dilutions, with inhibition remaining between the 1:5 and 1:25 dilutions for two of the samples. However, because the inhibition was minor for the inhibited soil samples (average ΔC_q=1.02) for the 1:5 dilution, and some soil samples retained inhibition after a five-fold dilution, we decided to not to dilute samples prior to qPCR analysis in order to avoid false negatives by also diluted our target genes in samples.

Table A.1. Primers and probes used for qPCR in this study.

Target Organism	Target gene	Primer/Probe Name	Sequence (5'-3')	Amplicon size (bp)	Reference
Adenovirus	Hexon	JTVXF	GGACGCCTCGGAGTACCTGAG	96	2,3
		JTVXR	ACIGTGGGGTTTCTGAACTTGTT		
		JTVXP	FAM-CTGGTGCAG/ZEN/TTCGCCCGTG CCA-IBFQ		
Campylobacter jejuni	ciaB	ciaB_718F	GCGTTTTGTGAAAAAGATGAAGATAG	77	4
		ciaB_797R	GGTGATTTTACTTTCATCCAAGC		
		UPL #137	Roche Universal Probe Library #137		
Shigella spp./EIEC	ipaH	ipaH_1136F	AAGGCCTTTTCGATAATGATACC	67	4
		ipaH_1202R	ATTTCGAGGCGGAACATTT		
		UPL #108	Roche Universal Probe Library #108		
Vibrio cholerae	ctxA	ctxA_F	TTTGTTAGGCACGATGATGGAT	84	4,5
		ctxA_R	ACCAGACAATATAGTTTGACCCACTAAG		
		ctxA probe	FAM-TGTTTCCAC/ZEN/CTCAATTAGTTT GAGAAGTGCCC-IBFQ		

Table A.2. Standards used for qPCR in this study.

Target Organism	Target gene	Standard type	Sequence (5'-3') or plasmid source	Size (bp)
Adenovirus	Hexon	DNA oligo	GGACGCCTCGGAGTACCTGAGCCCCGGGCTGG TGCAGTTTGCCCGCGCCACCGAGACGTACTTCA GCCTGAATAACAAGTTTAGAAACCCCACGGT	96
Campylobacter jejuni	ciaB	plasmid	Received from S. Ishii (University of Minnesota)[4]	4008
Shigella spp./EIEC	ipaH	plasmid	Received from S. Ishii (University of Minnesota)[4]	3997
Vibrio cholerae	ctxA	plasmid	Received from S. Ishii (University of Minnesota)[4]	4015

Table A.3. Limits of detection and quantification of *E. coli* and pathogen assays.

Sample location	*E. coli* CFU		Pathogen gene copies	
	LOD	ULOQ	LOD	LLOQ
Water (per 100 ml)	1 (0 log)	500 (2.70 log)	60 (1.78 log)	120 (2.08 log)
Hands (per 2 hands)	15 (1.18 log)	7,500 (3.88 log)	450 (2.65 log)	900 (2.95 log)
Surfaces (per 10cm x 10 cm area)	1 (0 log)	500 (2.70 log)	350 (2.54 log)	700 (2.85 log)
Soil (per 1 gram dry)	2,838 (3.45 log)	1,419,000 (6.15 log)	732 (2.86 log)	1,464 (3.17 log)
Drains/standing water (per 1 ml)	1,000 (3 log)	500,000 (5.70 log)	300 (2.48 log)	600 (2.78 log)
Streams (per 1 ml)	10,000 (5 log)	500,000,000 (8.70 log)	15-300 (2.35 - 2.47 log)	30-600 (1.48 – 2.78 log)

LOD = limit of detection, LLOQ = lower limit of quantification, ULOQ = upper limit of quantification
No ULOQ is reported for pathogens, because no pathogens were above the limit of quantification.

Table A.4. Means and standard deviations of log-transformed counts for *E. coli* and pathogens.

	E. coli	Adenovirus	*C. jejuni*	*Shigella* spp./EIEC	*V. cholerae*
Means for all samples assayed for each assays					
Water	0.60 (0.90)	1.49 (0.04)*	1.50 (0.07)	1.49 (0.04)*	1.53 (0.12)
Caregiver hands	2.64 (0.66)	2.66 (0.81)	2.37 (0.23)	2.50 (0.54)	2.34 (0.17)
Child hands	2.05 (0.79)	2.62 (0.77)	2.39 (0.33)	2.32 (0.16)	2.34 (0.13)
Tables	1.18 (0.88)	2.45 (0.49)	2.27 (0.08)	2.37 (0.38)	2.33 (0.14)
Plates	0.42 (0.75)	2.36 (0.31)	2.24 (0)*	2.28 (0.16)	2.30 (0.12)
Floors	1.82 (0.68)	2.65 (0.81)	2.49 (0.39)	2.35 (0.23)	2.29 (0.11)
Soil	5.55 (0.35)	3.18 (0.90)	3.31 (0.80)	3.55 (0.71)	2.63 (0.13)
Drains	4.98 (0.41)	2.96 (0.78)	2.65 (0.71)	2.99 (0.92)	2.22 (0.11)
Standing water	4.08 (1.00)	2.69 (0.44)	2.67 (0.25)	2.73 (0.42)	2.54 (0.13)
Streams	5.00 (0)**	3.50 (0.96)	2.78 (0.93)	3.94 (0.78)	1.90 (0.68)
Means only including samples above LOD for each pathogen and E. coli for samples positive for pathogens					
	E. coli	Adenovirus	*C. jejuni*	*Shigella* spp./EIEC	*V. cholerae*
Water	1.82 (0.61)	n/a	1.78 (0)	n/a	1.78 (0)
Caregiver hands	2.62 (0.67)	3.87 (0.96)	2.85 (0.28)	3.46 (0.68)	2.60 (0.12)
Child hands	2.25 (0.71)	3.99 (0.85)	2.96 (0.81)	2.65 (0)	2.50 (0.17)
Tables	1.25 (0.77)	3.26 (0.61)	2.54 (0)	3.30 (0.53)	2.54 (0)
Plates	0.94 (0.78)	3.11 (0.08)	n/a	2.85 (n/a)	2.54 (0)
Floors	1.92 (0.63)	3.32 (1.03)	2.83 (0.42)	2.66 (0.29)	2.54 (0)
Soil	5.61 (0.24)	3.78 (0.94)	3.52 (0.78)	3.72 (0.62)	2.87 (0.02)
Drains	5.14 (0.24)	3.46 (0.57)	2.94 (0.78)	3.49 (0.83)	2.48 (0)
Standing water	4.81 (0.31)	2.78 (0)	2.88 (0.20)	3.23 (0.39)	2.78 (0)
Streams	5.00 (0)**	3.84 (0.84)	3.44 (0.12)	3.95 (0.78)	2.48 (n/a)

* No positive samples were detected, so the mean reported is half of the LOD. There is a small SD for the water samples because one water sampled was turbid and less water was filtered, raising the LOD for that sample.

** Measurement for the only stream sample that was diluted enough to get quantifiable readings.

Table A.5. Significant associations ($p<0.05$) among transmission pathways shown in Figure 2.2 for adenovirus (A), *C. jejuni* (C), *Shigella* spp./EIEC, *V. cholerae* (V), at least one pathogen (1), and multiple pathogens (M) for three separate statistical tests.

Pathway	Statistical Test 1 (Fisher's exact test, presence/absence)*	Statistical Test 2 (T-test, presence/absence)**	Statistical Test 3 (T-test, presence increase log gene copies)***
Chickens-Soil	-	C	C
Chickens-Floor	C	C	C
Soil-Floor	C	A,C,S	C,S
Soil-All Hands	A	A,S	A,S
Soil-Caregiver hands	-	A	A
Soil-Child hands	-	C,M	-
Soil-Open drainage ditch/standing water	-	A,S	-
Open drainage ditch-hands	-	A,C,M	A
Floor-hands	A	A	A
Table-hands	A,S	A,S,1	A,V
Caregiver hands-child hands (Additional test results in Table 2.2)	A,1,M	A,1	A
Hands-stored drinking water	1	-	-

* Fisher's exact test to evaluate if there is a significant association between a pathogen being present at one exposure point and a pathogen being present at another exposure point.

** T-test to evaluate if there is a significant association between a pathogen being present at one exposure point and a pathogen being present at another exposure point.

*** T-test to evaluate if there is a significant association between a pathogen being present at one exposure point and the log gene copies of a pathogen at another exposure point.

Table A.6. Correlations between Log *E. coli* CFU and pathogen detection and counts for different exposure points/sampling media.

Pathogen measurement	Water	Hands	Surfaces	Soil	Drains/standing water/stream
Pathogen detected	No: 0.92 (0.68) Yes: 1.82 (0.61) t=-2.92, p=0.012	No: 2.47 (0.70) Yes: 2.49 (0.69) t=-0.13, p=0.447	No: 1.23 (0.77) Yes: 1.53 (0.80) t=-1.46, p=0.075	All but one sample had a pathogen	No: 4.34 (0.23) Yes: 5.14 (0.24) t=-4.34, p=0.038
Multiple pathogens detected	No samples had multiple pathogens	No: 2.41 (0.68) Yes: 3.00 (0.58) t=-2.66, p=0.012	No: 1.27 (0.78) Yes: 1.96 (0.61) t=-3.32, p=0.002	No: 4.99 (0.78) Yes: 5.61 (0.24) t=-1.10, p=0.234	No: 4.67 (0.41) Yes: 5.19 (0.26) t=-2.30, p=0.037
Log gene copies Adenovirus	Pearson's r = -0.226 p=0.277	Pearson's r = 0.119 p=0.345	Pearson's r = 0.304 p=0.016	Pearson's r = 0.029 p=0.904	Pearson's r = 0.427 p=0.219
Log gene copies *C. jejuni*	Pearson's r = 0.078 p=0.712	Pearson's r = 0.316 p=0.010	Pearson's r = 0.287 p=0.022	Pearson's r = 0.312 p=0.181	Pearson's r = 0.609 p=0.062
Log gene copies *Shigella* spp./EIEC	Pearson's r = -0.226 p=0.277	Pearson's r = 0.321 p=0.009	Pearson's r = 0.126 p=0.326	Pearson's r = 0.424 p=0.062	Pearson's r = 0.624 p=0.054
Log gene copies *V. Cholerae*	Pearson's r = 0.305 p=0.139	Pearson's r = 0.143 p=0.256	Pearson's r = -0.181 p=0.155	Pearson's r = 0.506 p=0.023	Pearson's r = -0.060 p=0.869

A.2 References

1. Cao, Y., Griffith, J. F., Dorevitch, S. & Weisberg, S. B. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *J. Appl. Microbiol.* 113, 66–75 (2012).
2. Ishii, S. *et al.* Microfluidic Quantitative PCR for Simultaneous Quantification of Multiple Viruses in Environmental Water Samples. *Appl. Environ. Microbiol.* 80, 7505–7511 (2014).
3. Jothikumar, N. *et al.* Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Appl. Environ. Microbiol.* 71, 3131–3136 (2005).
4. Ishii, S., Segawa, T. & Okabe, S. Simultaneous Quantification of Multiple Food and Waterborne Pathogens by Use of Microfluidic Quantitative PCR. *Appl. Environ. Microbiol.* AEM.00205-13 (2013). doi:10.1128/AEM.00205-13
5. Blackstone, G. M. *et al.* Use of a real time PCR assay for detection of the ctxA gene of Vibrio cholerae in an environmental survey of Mobile Bay. *J. Microbiol. Methods* 68, 254–259 (2007).

APPENDIX B: SUPPLEMENTAL INFORMATION FOR CHAPTER 3

MICROBIAL SOURCE TRACKING OF YOUNG CHILDREN'S FECES: EVALUATION OF METHODS AND ENVIRONMENTAL FECAL CONTAMINATION IN KENYA

B.1 Methods

Human-specific *Bacteroides* detection

qPCR was used to analyze DNA extract for the human-associated *Bacteroides* fecal marker HF183, which is a human marker previously validated in Kenya.[1] Each reaction was 15 μL, completed in 384-well plate, and contained 1X final concentration of SYBR Green I dye master mix (Applied Biosystems, Waltham, MA) and 250 nM of each primer.[2] Samples were amplified on an Applied Biosystems 7900HT Fast Real-Time PCR System using thermocycle conditions of 2 min at 50°C, then 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 45 sec at 53°C, and 1 min at 60°C, followed by a dissociation curve analysis at 15 sec at 95°C, 20 sec at 60°C, and 15 sec at 95°C to determine the melting temperature of amplified sequences. A standard curve was generated using 10-fold serial dilutions (3 to 3×10^6 genes copies/μL) of the standard sequence (target synthesized DNA). All environmental samples were analyzed in duplicate, standard dilutions were analyzed in triplicate, and three no-template controls were included on each plate. In order to be considered detected, the target HF183 sequence had to have amplified with a melting temperature between 73°C and 76°C. Assuming the recommended theoretical minimum detection of 3 copies per PCR reaction,[3] the average limits of detection (LOD) were 60 gene copies per 100 mL for water samples, 225 gene copies per two hands, 350 gene copies per 100

cm^2 for surface samples, 732 gene copies per gram of dry soil, 300 gene copies per mL of drain and standing water samples, and 15-300 gene copies per mL of stream samples (depending on stream filtration volume). All extraction blank and no template control samples were negative. Linearity (R^2=0.99) and efficiency (e=103%) were within acceptable ranges.

PCR and Sample Preparation for 16S Sequencing

The V3-V4 (357F/805R primers; 5'-CCTACGGGNGGCWGCAG-3' and 5'-GACTACHVGGGTATCTAATCC-3') and V4 (515F/806R primers; 5'-GTGYCAGCMGCCGCGGTAA-3' and 5'-GGACTACNVGGGTWTCTAAT-3') variable regions of the 16S rRNA gene were amplified by a two-step PCR using a Fluidigm access array integrated fluidic circuit. Prior to amplification, DNA samples were measured on a Qubit (Life Technologies, Waltham, MA) and each sample was diluted to 2 ng/µl or lower concentration. Roche High Fidelity Fast Start Kit and 20x Access Array loading reaction were used to prepare a mastermix following Fluidigm protocols. The final concentration of primers in the PCR reaction was 50 nM. Sample and primers were loaded on a Fluidigm 48 by 48 Access Array integrated fluidic circuit (IFC) using an AX controller (Fluidigm Corporation, South San Francisco, CA). Once the IFC was loaded, a Fluidigm Biomark HD PCR machine was used for the thermocycling program without imaging, which had annealing temperatures of 55°C and 60°C and an extension temperature of 72°C. After amplification, Fluidigm Harvest Buffer was loaded to harvest PCR products. The harvested product was transferred to a 96 well plate and diluted for a second round of amplification using Illumina linkers and barcodes, which had 15 cycles with an annealing temperature 60°C and an extension temperature of 72°C. Products were pooled in equal amounts based on product concentration, size selected using a 2% agarose E-gel (Life Technologies), and

extracted from the gel using Qiagen gel extraction kit (Qiagen, Hilden, Germany). These size-selected products were then run on an Agilent Bioanalyzer as a quality control measure to confirm the profile and average size prior to MiSeq sequencing.

Table B.1. Average relative abundance (%) of genus-level taxa for young child and older child/adult fecal samples using V3-V4 and V4 region primer sets. The "Less Abundant Taxa" classification includes taxa with less than 2% average relative abundance among samples.

Genus	V3-V4 Region		V4 Region	
	Older child/adult	Young child	Older child/adult	Young child
Prevotella	17.6	21.0	13.6	16.2
Bacteroides	6.3	12.4	5.0	11.0
Faecalibacterium	8.9	9.1	9.4	10.0
Bifidobacterium	0.8	8.0	1.3	11.5
Escherichia-Shigella	0.4	7.6	0.4	6.4
Succinivibrio	5.2	1.2	5.8	1.4
Lactobacillus	0.2	4.0	0.2	3.8
Streptococcus	0.3	4.0	0.4	4.7
Ruminococcus	3.7	0.5	4.4	0.3
Veillonella	0.2	3.4	0.2	3.7
Campylobacter	0.1	2.6	0.09	1.9
Megasphaera	0.1	2.5	0.1	3.1
Alloprevotella	2.5	1.1	1.8	0.9
Eubacterium rectale group	2.4	0.7	3.0	1.0
Treponema	2.3	5.7×10^{-4}	2.0	0.001
Haemophilus	0.3	2.3	0.4	2.6
Sutterella	1.4	2.2	1.8	2.6
Rikenellaceae gut group	2.1	0.02	1.7	0.03
Clostridiales uncultured bacterium	1.9	0.02	2.2	0.01
Less Abundant Taxa	45.2	17.3	49.7	21.8

Table B.2. Sensitivity and specificity for identifying the dominant source of human fecal contamination including young child, older child/adult, latrine, and open drain sources using SourceTracker.

	Young child		Older child & adult		Latrine		Open drain	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
V3V4 primers, with soil assigned as a "source"								
All samples	100%	100%	100%	81%	46%	100%	100%	100%
Water samples	100%	100%	100%	92%	50%	100%	100%	100%
Soil samples	100%	100%	100%	78%	33%	100%	100%	100%
V4 primers, with soil assigned as a "source"								
All samples	100%	91%	100%	89%	46%	100%	100%	100%
Water samples	100%	88%	100%	93%	50%	100%	100%	100%
Soil samples	100%	100%	100%	78%	33%	100%	100%	100%
V3V4 primers, without soil assigned as a "source"								
All samples	71%	96%	33%	92%	38%	100%	100%	67%
Water samples	92%	94%	50%	89%	50%	100%	100%	88%
Soil samples	0%	100%	0%	100%	0%	100%	100%	0%
V4 primers, without soil assigned as a "source"								
All samples	71%	91%	0%	95%	38%	100%	100%	64%
Water samples	92%	88%	0%	93%	50%	100%	100%	84%
Soil samples	0%	100%	0%	100%	0%	100%	100%	0%

Table B.3. Sensitivity and specificity for identifying the dominant source of human fecal contamination using Bray-Curtis distance-based methods.

	Young child		Older child & adult		Latrine		Open drain	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
V3V4 primers (all sources)								
All samples	71%	100%	67%	95%	69%	100%	100%	76%
Water samples	92%	100%	100%	93%	90%	100%	100%	100%
Soil samples	0%	100%	0%	100%	0%	100%	100%	0%
V4 primers (all sources)								
All samples	76%	100%	67%	97%	69%	100%	100%	76%
Water samples	100%	100%	100%	96%	90%	100%	100%	100%
Soil samples	0%	100%	0%	100%	0%	100%	100%	0%
V3V4 primers (only young child and older child/adult sources)								
All samples	71%	100%	100%	71%				
Water samples	92%	100%	100%	92%				
Soil samples	0%	100%	100%	0%				
V4 primers (only young child and older child/adult sources)								
All samples	76%	100%	100%	76%				
Water samples	100%	100%	100%	100%				
Soil samples	0%	100%	100%	0%				

Table B.4. Sensitivity and specificity for identifying the presence/absence of human fecal contamination sources including young child, older child/adult, latrine, and open drain sources using SourceTracker.

	Young child		Older child & adult		Latrine		Open drain	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
V3V4 primers, with soil assigned as a "source"								
All samples	91%	72%	100%	58%	87%	100%	100%	94%
Water samples	89%	58%	100%	52%	100%	100%	100%	92%
Soil samples	100%	100%	100%	78%	33%	100%	100%	100%
V4 primers, with soil assigned as a "source"								
All samples	91%	72%	100%	56%	93%	100%	100%	91%
Water samples	89%	58%	100%	48%	100%	100%	100%	88%
Soil samples	100%	100%	100%	78%	67%	100%	100%	100%
V3V4 primers, without soil assigned as a "source"								
All samples	86%	72%	100%	58%	87%	100%	100%	48%
Water samples	89%	58%	100%	52%	100%	100%	100%	63%
Soil samples	75%	100%	100%	78%	33%	100%	100%	0%
V4 primers, without soil assigned as a "source"								
All samples	71%	91%	0%	95%	38%	100%	100%	64%
Water samples	92%	88%	0%	93%	50%	100%	100%	84%
Soil samples	0%	100%	0%	100%	0%	100%	100%	0%

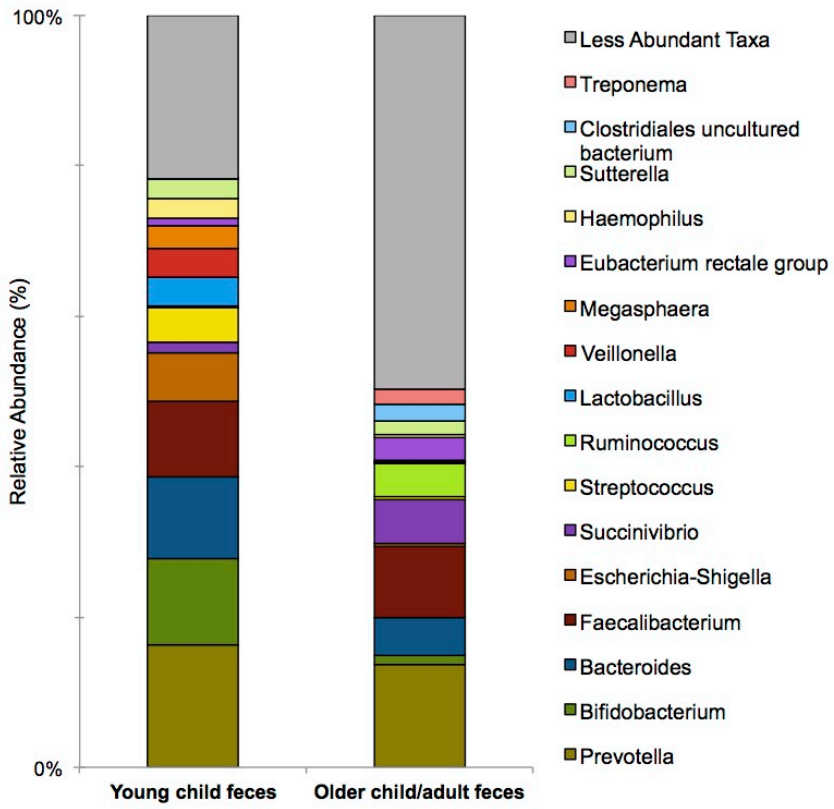


Figure B.1. Differences in the average relative abundance of genus-level taxa for young child and older child/adult fecal samples using the V4 region of the 16S rRNA gene primer set. The "Less Abundant Taxa" classification includes taxa with less than 2% average relative abundance among samples.

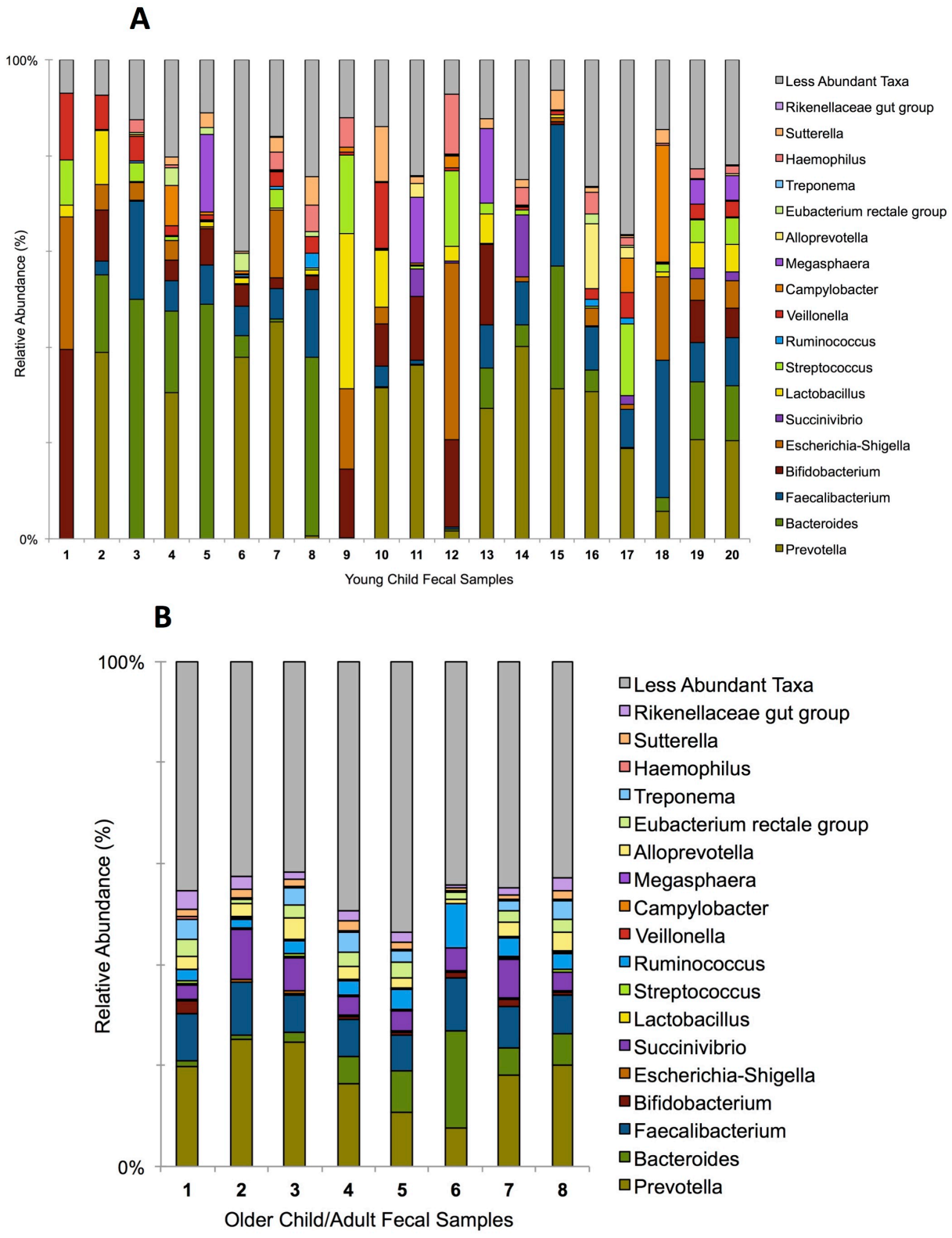


Figure B.2. Differences in the relative abundance of genus-level taxa for each young child (A) and older child/adult (B) fecal sample using V3-V4 region primer set. For young child fecal

samples, sample 1 is from a child 3 months old, sample 2 is from a child 9 months old, samples 3-10 are from children 12 to <18 months, samples 11-16 are from children 18 to <24 months, samples 17-18 are from children 24 to <36 months, and samples 19-20 are composite samples. For older child/adult, samples 1-6 are individual fecal samples, and samples 7-8 are composite samples. The “Less Abundant Taxa” classification includes taxa with less than 2% average relative abundance among all samples (excluding composite samples).

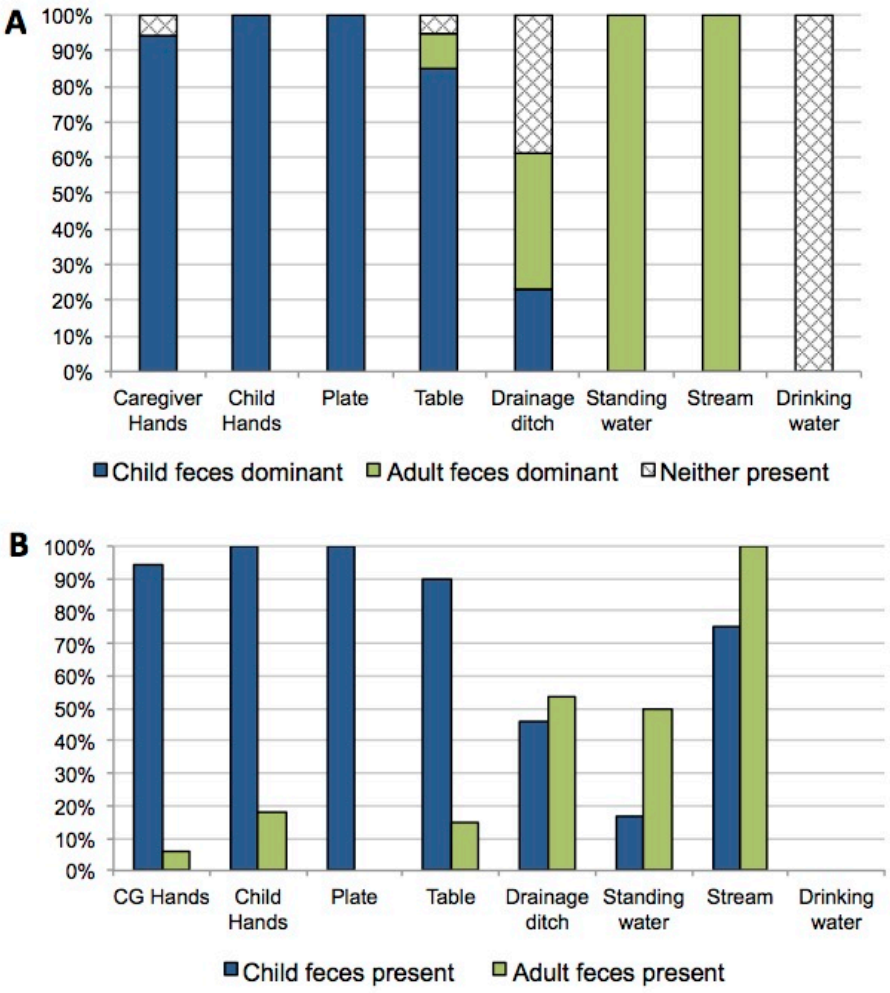


Figure B.3. Environmental sample results for (A) dominant human-associated source and (B) presence/absence of contamination source among samples that tested positive for the HF183 human fecal marker using V4 region primer set.

B.2 References

1. Jenkins, M. W., Tiwari, S., Lorente, M., Gichaba, C. M. & Wuertz, S. Identifying human and livestock sources of fecal contamination in Kenya with host-specific Bacteroidales assays. *Water Res.* 43, 4956–4966 (2009).
2. Seurinck, S., Defoirdt, T., Verstraete, W. & Siciliano, S. D. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environ. Microbiol.* 7, 249–259 (2005).
3. Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55, 611–622 (2009).

APPENDIX C: SUPPLEMENTAL INFORMATION FOR CHAPTER 4

THE EFFECT OF YOUNG CHILDREN'S FECES DISPOSAL PRACTICES ON CHILD GROWTH: EVIDENCE FROM 34 COUNTRIES

C.1 Methods

International Wealth Index (IWI)

The IWI is an index of household economic wealth that is based on asset ownership and can be used for wealth comparison among low- and middle-income countries (1). This wealth index is similar to the DHS wealth index included in DHS datasets, but is a measure of absolute wealth that is comparable across countries whereas the DHS wealth index is survey-specific and therefore only allows comparison of the relative level of wealth of households within the same country. The IWI used in this analysis was calculated based on the following aspects: floor material, number of household rooms, access to electricity, and ownership of assets including a television, refrigerator, phone, car, bicycle, cheap utensil (such as a radio or watch), and expensive utensil (such as an air conditioner or a computer). The IWI was calculated using a reduced formula that removes water and sanitation access from the score since these are included as separate variables in our model. However, even without water and sanitation facility information included in the IWI calculation, the reduced wealth index is still considered a good indicator of household wealth that is highly correlated with IWI (1).

Dietary Diversity Indicator Score

Dietary diversity may reflect a child's diet quality and nutrient intake and has been previously correlated with nutritional status (2). This variable was excluded from the final models because

dietary data was not available for 30% of children included in our study, but separate models were run for the subset of children with dietary information to evaluate if including this variable would have substantially affected the results of our model. A dietary diversity indicator score was calculated using a method modified from Arimond and Ruel (2), as a measure of each child's diet quality based on the diversity of foods consumed in the previous 24 hours. Scores between 0 and 7 were calculated by grouping food into categories and adding 1 point to the score for each of the following categories from which food were consumed: (a) bread, noodles, potatoes, cassava, other tubers, other made from grains; (b) beans, peas, lentils, nuts; (c) cheese, yogurt, other milk products; (d) meat, liver, heart, other organs, fish or shellfish; (e) vitamin A rich foods (including pumpkin, carrots, squash, dark green leafy vegetables, mangoes, papayas); and (f) other fruits and fruit juices not rich in vitamin A. Breastfeeding was also included in the dietary diversity indicator score as follows: 7 points given (maximum possible score) for breastfed children less than 6 months old, 4 points given for children 6-11 months, 2 points given for children 12-23 months, and 1 point for children 24 months or older.

Pooled models were reanalysed for all anthropometric outcomes in each of the three household-level models for all children with the dietary diversity indicator score, which included 140,972 children under five. There were no changes in the associations of anthropometric outcomes with child faeces disposal in models with the dietary score included and minimal changes to the size of the effect. The magnitude of all adjusted prevalence ratios in models with dietary score included were within 1% of the magnitude of these values for the same models without dietary scores and regression coefficients in models with dietary score included were within 0.01 of coefficient values for the same models without dietary scores.

Table C.1. Country-specific characteristics of children under five years of age included in analysis.

Country	Year(s)	Sample Size	Prevalence (%)			Toilet facility[a] (%)		Child faeces disposal[b] (%)	
			Stunting	Underweight	Wasted	Unimproved	Improved	Unimproved	Improved
Sub-Saharan Africa (SSA)									
Benin	2011-2012	7,533	44.0	21.4	16.3	11.3	25.5	11.7	19.9
Burkina Faso	2010	6,280	34.4	25.0	15.2	6.0	26.2	4.8	16.7
Burundi	2010	3,150	56.1	27.8	6.0	56.0	41.0	44.5	34.3
Cameroon	2011	4,662	32.0	13.8	5.6	41.1	51.5	30.8	43.6
Cote d'Ivoire	2011-2012	2,878	29.5	14.6	6.9	15.6	43.7	11.9	30.9
Dem. Republic of Congo	2013-2014	7,615	44.4	23.3	8.0	47.8	36.9	36.2	27.3
Ethiopia	2011	8,961	42.7	30.3	11.7	32.8	16.2	21.4	10.8
Gambia	2013	3,003	25.7	18.0	11.8	48.1	48.7	43.6	39.5
Ghana	2014	2,622	19.3	11.0	5.0	8.5	59.4	5.3	25.1
Guinea	2012	2,963	30.6	17.7	9.9	43.7	39.1	29.6	32.2
Kenya	2014	8,611	27.0	13.5	5.2	35.0	41.4	33.5	35.8
Lesotho	2009	1,509	39.7	13.5	4.6	20.6	27.5	17.6	21.5
Liberia	2013	3,082	30.9	15.4	6.7	8.4	32.5	8.9	12.5
Madagascar	2008-2009	4,754	48.6	n/a	n/a	38.6	5.6	28.6	3.6
Mali	2012-2013	4,280	37.7	25.2	12.6	47.8	41.7	26.9	32.6
Mozambique	2011	8,818	39.5	13.0	5.2	38.8	24.6	36.5	18.7
Namibia	2013	1,402	22.6	13.6	8.0	4.7	38.5	4.2	17.2
Niger	2012	4,653	42.0	35.6	18.3	8.3	23.8	5.2	19.3
Nigeria	2008	17,549	42.4	24.3	14.4	17.4	48.5	15.4	38.1
Rwanda	2010	3,704	43.8	11.3	2.8	24.0	75.0	21.7	68.2
Senegal	2014	5,224	21.7	14.6	6.7	32.7	45.1	25.3	41.4
Sierra Leone	2013	3,889	37.5	16.0	9.5	30.4	48.0	36.6	42.9
Tanzania	2010	5,518	40.4	17.0	6.1	59.6	21.2	55.2	18.1
Togo	2013-2014	3,087	28.2	16.8	7.3	8.3	28.1	6.6	20.9
Uganda	2011	1,872	31.7	13.2	5.0	50.0	36.1	47.2	32.6
Zambia	2007	4,515	43.8	14.1	5.8	44.3	30.0	42.3	26.3
Zimbabwe	2010-2011	4,164	31.6	10.0	3.4	10.0	54.0	12.9	47.5

Table C.1. (cont.)

Asia and North Africa (ANA)									
South Asia									
India	2005-2006	37,694	44.1	37.4	18.0	7.3	47.2	3.8	24.7
Nepal	2011	2,138	42.5	30.1	10.9	9.2	46.0	7.8	31.0
North Africa									
Egypt	2014	12,803	19.8	6.2	10.5	5.6	94.3	3.2	55.2
Caucasus and Central Asia									
Armenia	2010	1,309	21.1	4.4	4.1	19.0	81.1	12.8	68.6
Kyrgyz Republic	2012	3,800	18.1	3.6	2.9	1.8	98.2	0.8	57.7
Tajikistan	2012	4,301	25.0	12.0	10.0	3.9	95.6	3.1	83.9
Southeastern Asia									
Cambodia	2014	4,271	32.6	23.5	9.7	1.0	52.9	1.7	32.0

[a] Improved toilet: flush/pour flush toilet to a piped sewer system, septic tank, or pit latrine, VIP latrine, pit latrine with a slab, or composting toilet. Unimproved toilet: flush/pour flush toilet to somewhere else, pit latrine without a slab, bucket toilet, or hanging toilet/latrine.

[b] Improved child faeces disposal: child used or faeces put in an improved toilet; Unimproved disposal: child used or faeces put in an unimproved toilet; Unhygienic disposal: faeces left in open, buried, put/rinsed in a drain or ditch, or thrown in garbage. Unimproved disposal percentage is slightly higher than the percentage of unimproved toilets in some countries, because some households reported that child faeces was disposed of in a toilet or latrine but adults did not use a toilet facility. Classifying these households as unhygienic disposal instead of unimproved would not have affected the model conclusions observed for associations of anthropometric outcomes with child faeces disposal.

Table C.2. Unadjusted prevalence ratios (PRs) and 95% confidence intervals for stunting, severe stunting, underweight, severe underweight, wasting, and severe wasting due to improved child faeces disposal practices in pooled samples. Models include country fixed-effects.

	Model 1: All children included in model			Model 2: Only children in households with improved sanitation included in model		
	PR	95% CI	p-value	PR	95% CI	p-value
Stunting						
Children under 5	0.76	0.74 – 0.77	<0.001	0.89	0.86 – 0.91	<0.001
Children under 2	0.81	0.79 – 0.84	<0.001	0.97	0.93 – 1.01	0.118
Geographic area (children under 5)						
Sub-Saharan Africa	0.77	0.75 – 0.78	<0.001	0.89	0.86 – 0.93	<0.001
Asia & North Africa	0.74	0.72 – 0.76	<0.001	0.88	0.85 – 0.91	<0.001
Severe Stunting						
Children under 5	0.66	0.64 – 0.68	<0.001	0.81	0.77 – 0.84	<0.001
Children under 2	0.78	0.74 – 0.81	<0.001	0.97	0.91 – 1.04	0.378
Geographic area (children under 5)						
Sub-Saharan Africa	0.70	0.67 – 0.72	<0.001	0.86	0.81 – 0.91	<0.001
Asia & North Africa	0.58	0.55 – 0.62	<0.001	0.76	0.71 – 0.81	<0.001
Underweight						
Children under 5	0.66	0.65 – 0.68	<0.001	0.82	0.79 – 0.85	<0.001
Children under 2	0.68	0.65 – 0.71	<0.001	0.84	0.79 – 0.89	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.69	0.67 – 0.72	<0.001	0.83	0.79 – 0.88	<0.001
Asia & North Africa	0.62	0.60 – 0.65	<0.001	0.80	0.77 – 0.84	<0.001
Severely Underweight						
Children under 5	0.55	0.52 – 0.58	<0.001	0.74	0.69 – 0.80	<0.001
Children under 2	0.61	0.56 – 0.66	<0.001	0.82	0.73 – 0.91	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.61	0.57 – 0.66	<0.001	0.78	0.70 – 0.87	<0.001
Asia & North Africa	0.47	0.43 – 0.51	<0.001	0.72	0.65 – 0.79	<0.001
Wasting						
Children under 5	0.77	0.74 – 0.80	<0.001	0.88	0.83 – 0.92	<0.001
Children under 2	0.78	0.74 – 0.82	<0.001	0.93	0.87 – 0.995	0.035
Geographic area (children under 5)						
Sub-Saharan Africa	0.79	0.75 – 0.83	<0.001	0.86	0.79 – 0.93	<0.001
Asia & North Africa	0.74	0.70 – 0.78	<0.001	0.89	0.83 – 0.95	0.001
Severe Wasting						
Children under 5	0.78	0.73 – 0.83	<0.001	0.86	0.79 – 0.93	<0.001
Children under 2	0.82	0.76 – 0.89	<0.001	0.93	0.83 – 1.04	0.209
Geographic area (children under 5)						
Sub-Saharan Africa	0.80	0.74 – 0.87	<0.001	0.86	0.75 – 0.98	0.022
Asia & North Africa	0.74	0.68 – 0.82	<0.001	0.86	0.77 – 0.95	0.005

Table C.3. Unadjusted regression coefficients and 95% confidence intervals for height-for-age, weight-for-age, and weight-for-height z-scores due to improved child faeces disposal practices in pooled samples. Models include country fixed-effects.

	Model 1: All children included in model			Model 2: Only children in households with improved sanitation included in model		
	Coef.	95% CI	p-value	Coef.	95% CI	p-value
Height-for-age z score						
Children under 5	0.32	0.30 – 0.34	<0.001	0.08	0.05 – 0.11	<0.001
Children under 2	0.23	0.20 – 0.27	<0.001	0.03	-0.02 – 0.08	0.202
Geographic area (children under 5)						
Sub-Saharan Africa	0.33	0.31 – 0.36	<0.001	0.09	0.05 – 0.14	<0.001
Asia & North Africa	0.29	0.25 – 0.33	<0.001	0.07	0.03 – 0.12	0.001
Weight-for-age z score						
Children under 5	0.28	0.26 – 0.29	<0.001	0.07	0.05 – 0.10	<0.001
Children under 2	0.25	0.23 – 0.28	<0.001	0.05	0.02 – 0.09	0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.27	0.25 – 0.29	<0.001	0.09	0.05 – 0.12	<0.001
Asia & North Africa	0.28	0.26 – 0.31	<0.001	0.06	0.03 – 0.09	<0.001
Weight-for-height z score						
Children under 5	0.12	0.10 – 0.13	<0.001	0.02	-0.01 – 0.04	0.236
Children under 2	0.15	0.12 – 0.18	<0.001	0.01	-0.03 – 0.05	0.494
Geographic area (children under 5)						
Sub-Saharan Africa	0.10	0.08 – 0.12	<0.001	0.03	-0.01 – 0.06	0.166
Asia & North Africa	0.15	0.12 – 0.18	<0.001	0.01	-0.03 – 0.04	0.696

Table C.4. Adjusted regression coefficients and 95% confidence intervals for height-for-age, weight-for-age, and weight-for-height z-scores due to improved child faeces disposal practices in pooled samples only including households with improved sanitation.

	Model 3: Improved child faeces disposal			Model 3: Unimproved child faeces disposal		
	Coef.	95% CI	p-value	Coef.	95% CI	p-value
Height-for-age z score						
Children under 5	0.12	0.10 – 0.14	<0.001	-0.01	-0.04 – 0.01	0.268
Children under 2	0.13	0.09 – 0.17	<0.001	0.00	-0.04 – 0.04	0.938
Weight-for-age z score						
Children under 5	0.09	0.08 – 0.11	<0.001	0.04	0.02 – 0.06	<0.001
Children under 2	0.09	0.07 – 0.12	<0.001	0.02	-0.01 – 0.05	0.107
Weight-for-height z score						
Children under 5	0.02	0.00 – 0.04	0.096	0.05	0.03 – 0.08	<0.001
Children under 2	0.03	0.00 – 0.06	0.067	0.03	-0.01 – 0.06	0.142

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects.

Table C.5. Urban and rural comparison for adjusted prevalence ratios and 95% confidence intervals for stunting, underweight, and wasting due to improved child faeces disposal practices in pooled samples (Model 1 characteristics).

	Urban			Rural		
	aPR	95% CI	p-value	aPR	95% CI	p-value
Stunting						
Child age						
Children under 5	0.92	0.89 – 0.95	<0.001	0.93	0.91 – 0.95	<0.001
Children under 2	0.90	0.85 – 0.94	<0.001	0.93	0.89 – 0.96	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.95	0.91 - 0.99	0.011	0.95	0.92 – 0.97	<0.001
Asia & North Africa	0.88	0.84 – 0.92	<0.001	0.88	0.85 – 0.92	<0.001
Underweight						
Child age						
Children under 5	0.88	0.84 – 0.92	<0.001	0.89	0.86 – 0.92	<0.001
Children under 2	0.86	0.80 – 0.92	<0.001	0.88	0.83 – 0.93	<0.001
Geographic area						
Sub-Saharan Africa	0.91	0.86 – 0.97	0.006	0.92	0.89 – 0.97	<0.001
Asia & North Africa	0.84	0.79 – 0.89	<0.001	0.81	0.76 – 0.85	<0.001
Wasting						
Child age						
Children under 5	0.93	0.88 – 0.99	0.026	0.97	0.92 – 1.03	0.325
Children under 2	0.92	0.85 – 0.997	0.041	0.96	0.90 – 1.03	0.249
Geographic area						
Sub-Saharan Africa	0.91	0.83 – 0.998	0.044	0.98	0.92 – 1.05	0.628
Asia & North Africa	0.93	0.86 – 1.01	0.095	0.92	0.84 – 1.02	0.104

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects.

Table C.6. Urban and rural comparison for adjusted regression coefficients and 95% confidence intervals for height-for-age, weight-for-age, and weight-for-height z-scores due to improved child faeces disposal practices in pooled samples (Model 1 characteristics).

	Urban			Rural		
	Effect	95% CI	p-value	Effect	95% CI	p-value
Height-for-age z score						
Child age						
Children under 5	0.10	0.06 – 0.14	<0.001	0.10	0.07 – 0.13	<0.001
Children under 2	0.12	0.07 – 0.18	<0.001	0.12	0.07 – 0.16	<0.001
Geographic area (children under 5)						
Sub-Saharan Africa	0.04	0.00 – 0.09	0.054	0.08	0.04 – 0.11	<0.001
Asia & North Africa	0.17	0.11 – 0.24	<0.001	0.13	0.08 – 0.19	<0.001
Weight-for-age z score						
Child age						
Children under 5	0.08	0.06 – 0.11	<0.001	0.06	0.04 – 0.08	<0.001
Children under 2	0.08	0.04 – 0.12	<0.001	0.08	0.04 – 0.11	<0.001
Geographic area						
Sub-Saharan Africa	0.04	0.01 – 0.08	0.013	0.04	0.01 – 0.07	0.003
Asia & North Africa	0.13	0.10 – 0.17	<0.001	0.09	0.06 – 0.13	<0.001
Weight-for-height z score						
Child age						
Children under 5	0.03	-0.01 – 0.06	0.101	-0.01	-0.04 – 0.02	0.468
Children under 2	0.02	-0.03 – 0.07	0.406	0.02	-0.03 – 0.06	0.469
Geographic area						
Sub-Saharan Africa	0.02	-0.02 – 0.06	0.347	-0.02	-0.05 – 0.02	0.332
Asia & North Africa	0.03	-0.02 – 0.08	0.200	0.01	-0.04 – 0.06	0.650

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects.

Table C.7. Adjusted prevalence ratios (aPRs) and 95% confidence levels for the association of community coverage levels with underweight and wasting.

	Model 7	Model 8	Model 9	Model 10	Model 11	Model 12
Underweight						
Community coverage of improved toilets						
25-50% vs. 0-25% coverage	0.95 (0.92-0.98)	0.96 (0.93-0.99)	0.95 (0.92-0.99)	-	-	-
50-75% vs. 0-25% coverage	0.90 (0.87-0.94)	0.93 (0.89-0.96)	0.94 (0.90-0.98)	-	-	-
75-100% vs. 0-25% coverage	0.85 (0.83-0.88)	0.89 (0.86-0.92)	0.88 (0.83-0.93)	-	-	-
Household improved toilet access (vs. unimproved or open defecation)	-	0.94 (0.92-0.97)	0.94 (0.90-0.99)	-	-	-
Improved toilet interaction terms						
Household improved toilet x 25-50% Community coverage	-	-	1.02 (0.95-1.10)	-	-	-
Household improved toilet x 50-75% Community coverage	-	-	0.98 (0.91-1.05)	-	-	-
Household improved toilet x 75-100% Community coverage	-	-	1.01 (0.94-1.09)	-	-	-
Community coverage of improved child faeces disposal						
25-50% vs. 0-25% coverage	-	-	-	0.91 (0.88-0.94)	0.92 (0.89-0.96)	0.92 (0.89-0.96)
50-75% vs. 0-25% coverage	-	-	-	0.89 (0.86-0.92)	0.92 (0.89-0.96)	0.93 (0.89-0.97)
75-100% vs. 0-25% coverage	-	-	-	0.85 (0.81-0.89)	0.90 (0.86-0.94)	0.87 (0.81-0.93)
Household improved child faeces disposal (vs. unimproved or unhygienic)	-	-	-	-	0.91 (0.88-0.93)	0.90 (0.85-0.94)
Improved child faeces disposal interaction terms						
Household disposal x 25-50% Community coverage	-	-	-	-	-	1.01 (0.94-1.09)
Household disposal x 50-75% Community coverage	-	-	-	-	-	0.99 (0.92-1.07)
Household disposal x 75-100% Community coverage	-	-	-	-	-	1.06 (0.97-1.16)
Wasted						
Community coverage of improved toilets						
25-50% vs. 0-25% coverage	0.95 (0.90-0.999)	0.96 (0.91-1.01)	0.98 (0.93-1.04)	-	-	-
50-75% vs. 0-25% coverage	0.90 (0.85-0.95)	0.93 (0.87-0.98)	0.91 (0.85-0.98)	-	-	-

Table C.7. (cont.)

75-100% vs. 0-25% coverage	0.90 (0.85-0.94)	0.94 (0.89-0.99)	0.89 (0.81-0.97)	-	-	-
Household improved toilet access (vs. unimproved or open defecation)	-	0.94 (0.90-0.97)	0.93 (0.86-1.00)	-	-	-
Improved toilet interaction terms						
Household improved toilet x 25-50% Community coverage	-	-	0.94 (0.84-1.05)	-	-	-
Household improved toilet x 50-75% Community coverage	-	-	1.03 (0.92-1.15)	-	-	-
Household improved toilet x 75-100% Community coverage	-	-	1.07 (0.96-1.20)	-	-	-
Community coverage of improved child faeces disposal						
25-50% vs. 0-25% coverage	-	-	-	0.90 (0.85-0.95)	0.90 (0.85-0.95)	0.92 (0.86-0.97)
50-75% vs. 0-25% coverage	-	-	-	0.88 (0.83-0.93)	0.88 (0.83-0.94)	0.86 (0.80-0.93)
75-100% vs. 0-25% coverage	-	-	-	0.95 (0.89-1.01)	0.95 (0.90-1.02)	0.92 (0.85-1.01)
Household improved child faeces disposal (vs. unimproved or unhygienic)	-	-	-	-	0.99 (0.95-1.03)	0.97 (0.90-1.04)
Improved child faeces disposal interaction terms						
Household disposal x 25-50% Community coverage	-	-	-	-	-	0.96 (0.86-1.07)
Household disposal x 50-75% Community coverage	-	-	-	-	-	1.07 (0.95-1.19)
Household disposal x 75-100% Community coverage	-	-	-	-	-	1.06 (0.94-1.19)

Models adjust for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card, and include country fixed-effects.

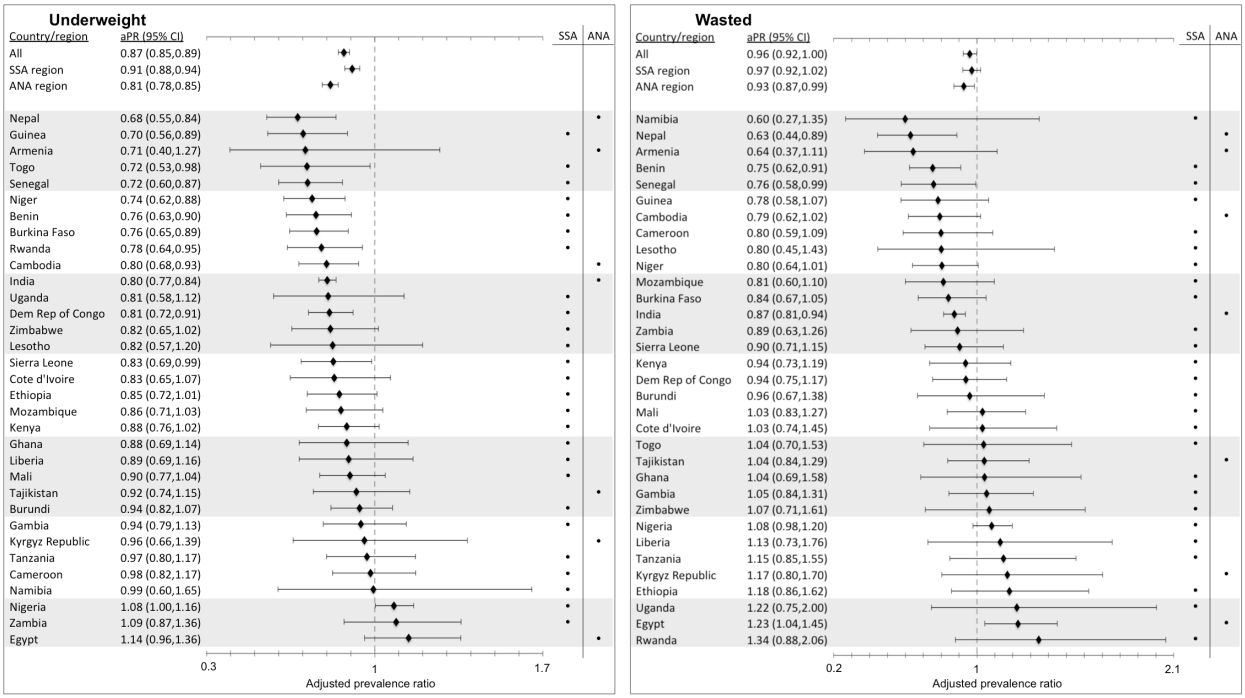
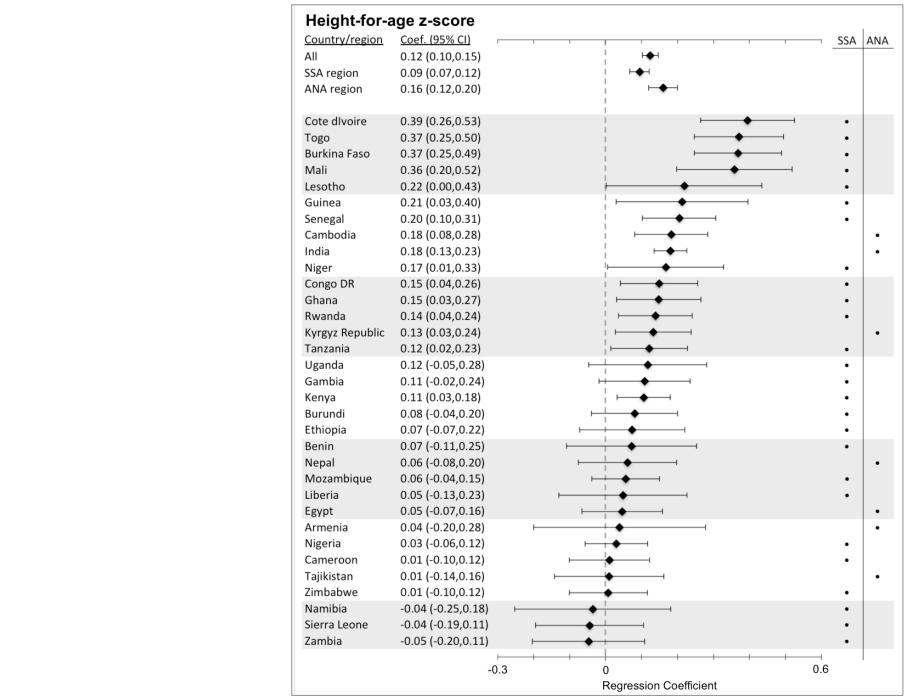


Figure C.1. Country-specific adjusted prevalence ratio (aPR) and 95% confidence intervals due to improved child faeces disposal practices for Model 1 characteristics, which includes all pooled households. Models have been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.



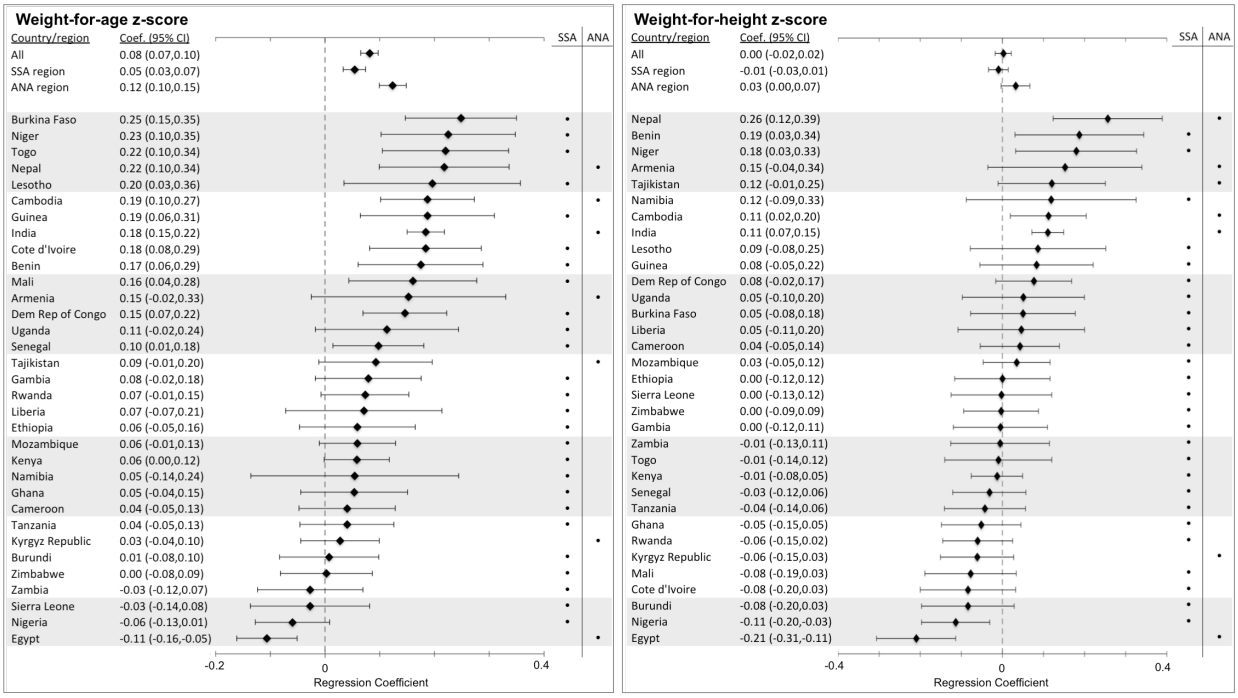
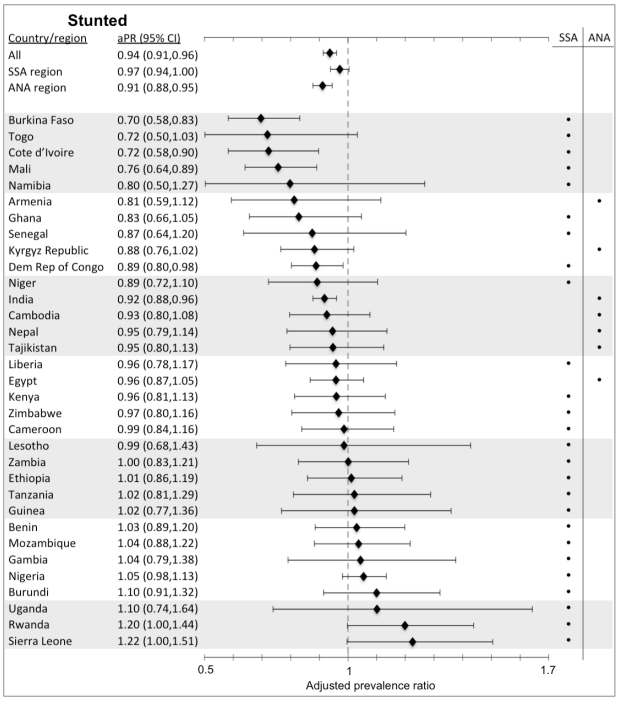
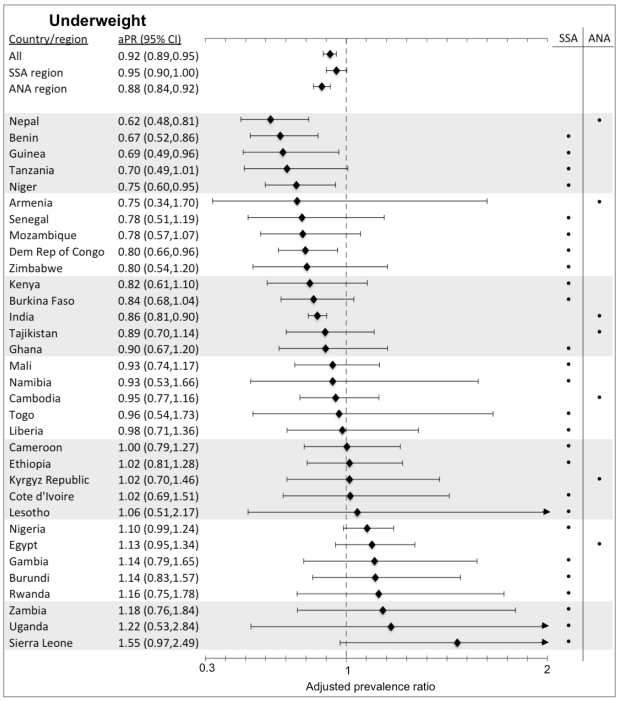


Figure C.2. Country-specific regression coefficients and 95% confidence intervals due to improved child faeces disposal practices using Model 1 characteristics, which includes all pooled households. Models have been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.





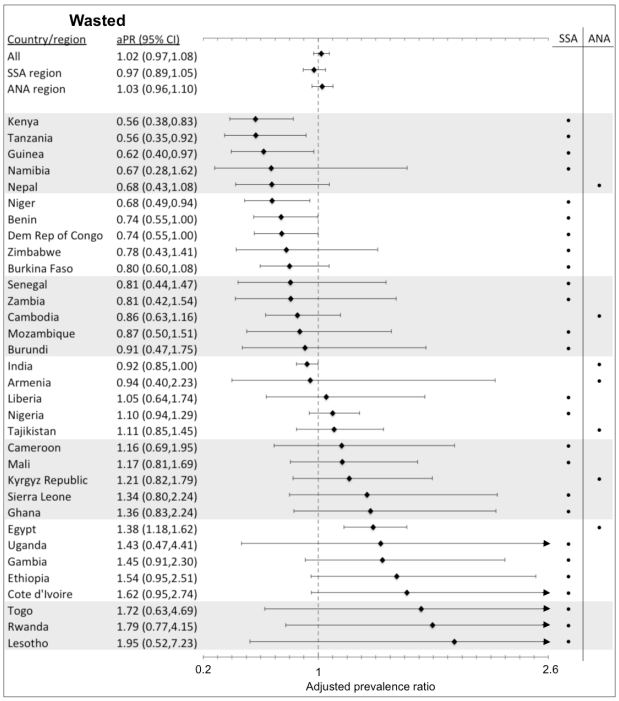


Figure C.3. Country-specific adjusted prevalence ratio (aPR) and 95% confidence intervals due to improved child faeces disposal practices using Model 2 characteristics, which only includes households with improved sanitation. Models have been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.

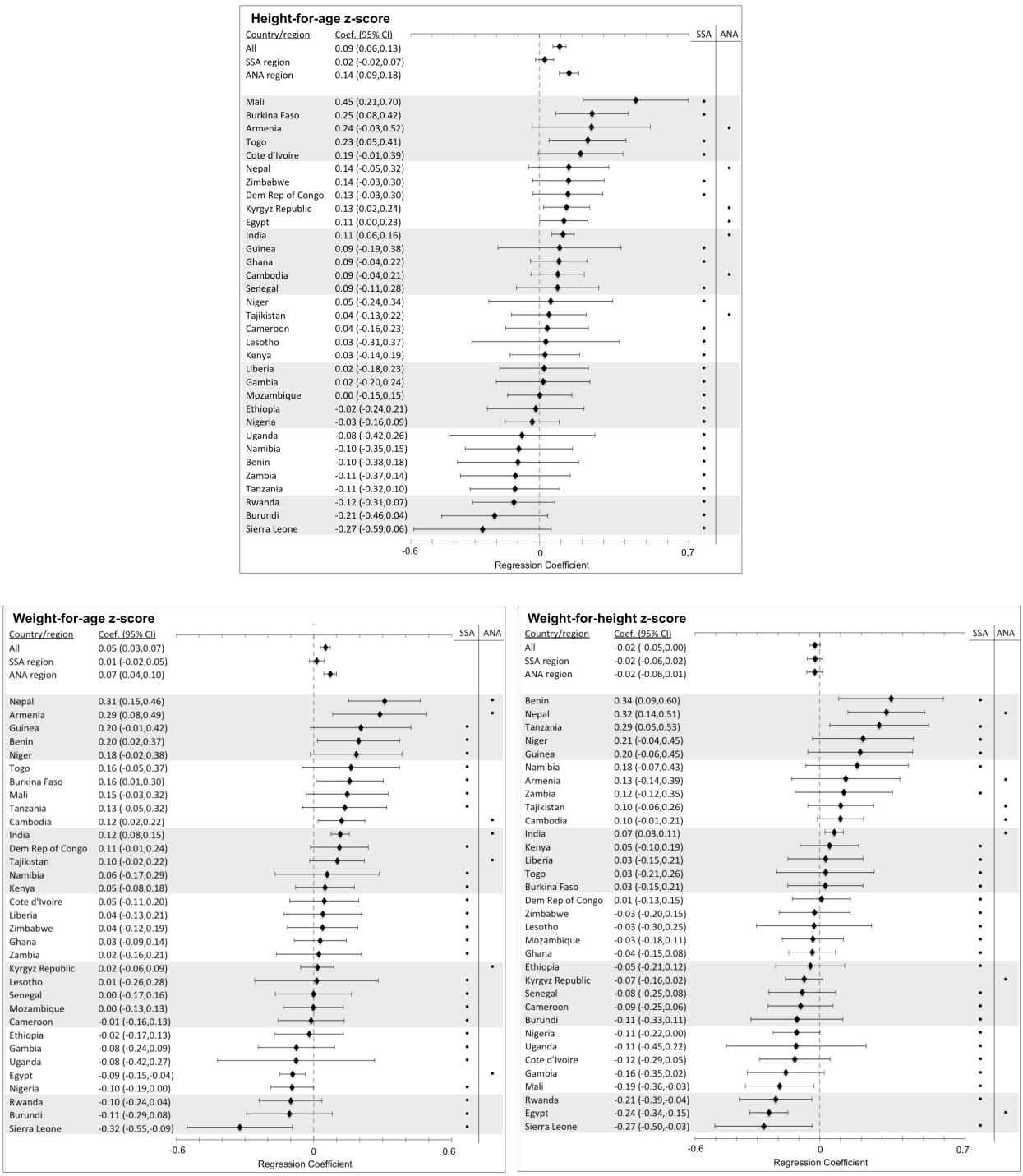


Figure C.4. Country-specific regression coefficients and 95% confidence intervals due to improved child faeces disposal practices using Model 2 characteristics, which only includes households with improved sanitation. Models have been adjusted for access to improved water, international wealth index, mother's education, child's age, mother's age, sex of child, marital status of mother, pregnancy status of mother, whether child is a twin, and whether the child has a health card.

C.2 References

1. Smits J, Steendijk R. The international wealth index (IWI). *Soc Indic Res* 2015; 122:65–85.
2. Arimond M, Ruel MT. Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health surveys. *J Nutr* 2004; 134:2579–2585.